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FLOWER AND SEED OF HEDYOSMUM NUTANS[1]

J. GRAHAM EDWARDS

(WITH PLATES XXXIV–XXXVI)

The investigation of the seed development of *Hedyosmum* (Chloranthaceae) was undertaken at the suggestion of Professor DUNCAN S. JOHNSON. Of the 20 or more species of this genus, two species were studied. These two, *H. nutans* and *H. arborescens*, represent the largest of the three subgenera of the genus, so that the following results can perhaps be taken as characteristic of the genus as a whole. All of the figures used, except fig. 38, are from *H. nutans*. In the important features of the development of the embryo sac, perianth, etc., of *H. arborescens*, there is quite close agreement with those of *H. nutans*.

All of the material used was fixed by JOHNSON in chromo-acetic fixative.[2] The further work of imbedding, sectioning, and staining was done by the writer, except that figs. 41 and 44 were made from slides previously prepared by JOHNSON. The sections from which drawings were made were cut 7 μ thick. All sectioned material was stained with Flemming's triple stain and mounted in balsam.

The writer gratefully acknowledges the aid received from Professor JOHNSON, who inspected the slides from which drawings were made, read the manuscript, and gave helpful suggestions.

[1] Botanical Contribution from the Johns Hopkins University, no. 65.

[2] The material used was collected and fixed by D. S. JOHNSON in Jamaica in 1906, with the aid of a grant from the Bache fund.

Staminate flowers

The staminate flowers arise in short conelike catkins, consisting of 100 or more flowers each, one male catkin arising beside the female inflorescence near the end of the flowering shoot. Each male flower consists solely of a single, elongated, inverted, pyramidal stamen with a microsporangium at each of its four corners.

The microsporangia possess the usual epidermis, endothecium, tapetum, and several-layered archesporium (figs. 1, 2). From the spore mother cells, tetrads of microspores are produced in the usual way (figs. 3–5). The outer wall of the microspores (exine) is roughened by closely packed peglike tubercles 1 μ long and 0.5 μ in diameter (fig. 5). There are also present over the surface of the microspore 6 unthickened meridional bands, through one of which the pollen tube emerges (fig. 6, polar view). The inner wall of the microspore (intine) consists of a darkly staining layer of cellulose (fig. 5). The mature pollen grain has the usual vegetative and generative nuclei (fig. 5).

CLARKE (**5**) describes the stamens of the genera *Chloranthus*, *Ascarina*, and *Hedyosmum* as being two-celled, and before opening four-celled, due to a spurious, not always complete, dissepiment in the line of dehiscence. He says:

> The question referring to the structure of the anthers appears to have arisen entirely from those of *Chloranthus* itself as those of the other genera [of the family Chloranthaceae] are all of the ordinary two-celled character, or are spuriously four-celled from induplication at the line of dehiscence, a common occurrence with two-celled anthers; and in fact the four-celled structure is more apparent on a cross-section being made, both in *Hedyosmum* and *Ascarina*, especially the latter.

Neither SOLMS (**12**), BAILLON (**1**), EICHLER (**6**), BENTHAM and HOOKER (**2**), VAN TIEGHEM (**13**), nor ENGLER (**7**) refer to such peculiarity of structure as that mentioned by CLARKE. They describe the anthers as being of the ordinary four-sporangiate type. The writer finds his results agreeing with the later descriptions. In the sections of young anthers there are four distinct sporangia present from the outset of development (fig. 1).

Carpellate flowers

The carpellate flowers occur in compact cymelike inflorescences, several cymes in the axil of each leaf of the flowering branch.

Each flower is subtended by a hood-shaped bract, in the axil of which it arises. This bract, in early development, completely incloses the carpel and perianth (figs. 8, 12, 24).

When first observed, the young female flower consists of a short oblique column of tissue which is triangular in cross-section. On the side directly opposite the midrib of the bract the flower is distinctly lower than the sides next to the bract (figs. 8, 10).

PERIANTH.—The first step in the differentiation of the flower is the appearance of a ringlike upgrowth of the marginal portions of each of the faces of the triangular flower rudiment. This upgrowth is the young perianth. In the earliest stages seen this ring is clearly higher on the two faces lying to the right and left of the midrib of the bract. At first the outline of the floral rudiment as seen in cross-section is convex all around (fig. 11). Somewhat later, however, the outer tissue of the rudiment, that is, the tissue of the perianth, becomes much thicker on the three angles of the young flower, and a distinct depression is evident midway of the length of the flower on each side between the two angles (figs. 9, 10 *Po*). The depressed area bounded by the perianth is the wall of the carpel (fig. 17). This growth of the perianth continues from all directions, until in the ripe seed the wall of the carpel is overgrown by the perianth except for a small pore in each of the three flat sides (cf. figs. 9, 10, 25).

The character of the growth of the perianth is very evident from cross-sections (figs. 11 [upper and lower flower], 18) and from longitudinal sections (figs. 12, 14, 22). Only along a narrow strip, up and down each of the three corners of the ovary (figs. 11 [*X*, upper flower], 12 *Y*), and at a likewise narrow circular band around the upper third of the ovary (fig. 22 *Y*), are the tissues of the carpel and perianth continuous with each other.

A delicate vascular bundle is differentiated in the perianth at each of its three corners (figs. 11, 16). This vascular bundle divides into two near the top of the perianth, and these branches turn one to the right and one to the left, and later are found to be joined to an offshoot of a bundle of the carpel that pushes out into the perianth through the zone of attachment near the top of the ovary (fig. 22). The point of emergence of this carpellary bundle

is directly above the pore in the perianth on the flat side of the flower.

The perianth is never conspicuous either for its size or color. It is greenish, slightly yellow, or brown. It contains on its corners 15–20 layers of cells. The cells remain parenchymatous at first, but in the ripe seed they have rather thick walls and dense contents. The cells bordering on the pore become increasingly cutinized as development progresses.

Earlier observations on this ovary are the following: CLARKE (5) describes the female flowers as consisting of three sepals forming a tube not for the most part adhering to the ovary except at its base and apex where it becomes trifid. He says:

> There is a peculiarity occurring in the calyx of *Hedyosmum hirsutum* or an allied species (one of those in which the flowers are inclosed within thickened bracts so compactly that the apex of the calyx and stigma are alone discernible) which is probably quite singular: on removing bracteae, it is found that the calyx does not completely cover the ovary, but has three large loopholes, as it were, so that three flattened sides of the ovary are seen through it, although it is quite continuous at the angles and crowns it with its three segments as in other species.

SOLMS speaks of a triangular ovary and of the perigon as trifid at the apex, with the stigma sometimes alternate with the lobes of the perigon. BAILLON makes the following observation concerning the perianth: "Moreover, the apex of the ovary bears three short, thick, rounded wings alternating with its angles, two anterior and one posterior. Their morphological value is still uncertain." EICHLER speaks of a three-lobed half, or quite superior perigon. BENTHAM and HOOKER describe the tube of the perianth as grown to the ovary. VAN TIEGHEM says: "La fleur femelle, également nue, se compose d'un seule carpelle à style court renfermant un ovule orthotrope pendant; autour de la base du style, la paroi de l'ovaire se renfle quelquefois en trois bosses épaisses (Hed.)." ENGLER describes briefly the female flower as almost tubular and trifid at the corners. CLARKE has given a fuller description of the perianth, and each observer has mentioned some of the facts.

The question now remains as to the morphological nature of the perianth. The occurrence of three vascular bundles in the

perianth at the corners of the ovary, alternating with the three vascular bundles of the ovary itself, suggests that the perianth may be composed of three modified stamens standing one at each angle of the ovary. There is no definite evidence in favor of this view, since the earliest phases of the developing perianth show no essential peculiarities common to the perianth and to the stamens. The difficulty in accepting this homology is found in the unusual development of the upper portion of the perianth, the tissue of which in the earliest observed stages was in direct continuity with that of the ovary. In favor of such a view as just mentioned, perhaps, is ENGLER'S statement that the stamens of *Chloranthus inconspicuus* Sw. are united with a somewhat similar structure extending upward beyond the ovary about one-half its circumference.

Here also must be mentioned the fact that EICHLER (**6**, p. 7, in fig. 3 *B* and *C*) shows in *Chloranthus inconspicuus* an additional structure outside the three stamens, which he designates as a perianth. He says: "Das unterständige Ovar trägt an dem der Braktee zugekehrten Rande ein kleines, mehr weniger herablaufendes Schüppchen, das gewöhnlich als Andeutung eines Perigons betrachtet wird, und innerhalb dieses ein der Axe zugekrümmtes grosses dreilappiges Gebilde, das Androecium." Which of the two structures mentioned compares most closely in origin with the perianth of *Hedyosmum* must be determined by a study of the development of these structures in *Chloranthus*.

It seems clear from the preceding that the origin and growth of the structure termed perianth in *Hedyosmum*, especially in the presence of the lateral pore, differs from that of the perianth of any other angiosperm so far as is known to the writer.

OVARY.—The initial stage in the development of the ovary is that of a convex mass of cells (fig. 7). The outer margin of this dome of cells soon begins to grow more actively, thus leaving a depression in the center of the originally convex mass of cells (fig. 8, flower on left). The rapidly upgrowing ring of cells forms the wall of the ovary, while the central depression which is thus left at length becomes the ovarian cavity (figs. 8, 13, 18). By the continued upgrowth of the carpellary tissue about this depression, the ovarian cavity becomes deepened (figs. 13, 14 *OvC*).

Meanwhile the single nucellus of the ovary grows downward from one side (fig. 14), thus making the ovarian cavity appear crescentic in its upper half when seen in cross-section (fig. 18). This cavity in the lower portion about the hanging micropylar end of the ovule is completely circular in form (fig. 11 *OvC*, upper flower).

With the further growth of the ovary the ovarian cavity is extended upward by the stylar canal which opens out on the side of the style opposite the subtending bract (figs. 14 *SyC*, 22). The lower part of this ovarian cavity at the time the embryo sac is mature extends downward as an inverted conical chamber for half the distance to the base of the ovary (fig. 22). During the maturing of the fruit the cavity is filled by the single seed (fig. 42).

The style and stigma are developed by the continued growth of the upper margin of the carpellary ring, after the latter has closed together above the ovule to form the distinct stylar canal (figs. 14, 22). The stigma is formed solely from the longer lip of the upgrowing carpel (fig. 22). It consists, at the time of pollination, of a flattened triangular surface, the cells of which are parenchymatous. Later, in the ripe fruit, the mature stigma shrivels and disappears. The style is rather short. Internally it consists of two or more layers of elongated cells of small diameter which border on the stylar canal and probably serve as conducting tissue for the pollen tube (fig. 22). Around these layers of conducting tissue are 15 layers of other cells.

Early in its development the wall of the ovary consists of about 10 layers of cells (fig. 14). At the time the embryo sac is ripe the ovarian wall opposite the pore of the perianth is often 15 cells in thickness. Of the 15 layers of cells constituting the ovarian wall, only the 7 outer ones are appreciably specialized in structure. The epidermis becomes gradually cutinized as the embryo sac matures, and papillose cells arise in the exposed area where the perianth does not at first cover the carpellary wall. The cutin layer on the walls of these cells is thickest opposite the pore in the perianth, and becomes gradually thinner in the epidermal cells farther away from it.

At this stage, also, the 6 layers of cells within the epidermis of the ovary are strikingly irregular in contour. The 3 layers immedi-

ately adjoining the epidermis possess irregular cell contours, but their walls are not thickened. They are narrow and elongated. In the nearly mature seed they are not distinguishable from the epidermis, which is non-staining and presents a gelatinous appearance. The next 3 layers of cells adjoining these are thin in radial direction, while tangentially and longitudinally they are of considerably greater dimensions. By far the most salient peculiarity of these cells is the uneven thickening of the walls, which, when first appreciably thickened, are 2–3 μ thick. In the mature seed they are 7–8 μ thick. In the latter case the entire cell cavity is occluded and there is no trace of a nucleus, whereas in the younger ovary, with slightly thickened walls, the nuclei are distinct and remain so until the 10-celled stage of the embryo. The uneven thickening is shown in places by relatively large pits or thin areas (fig. 23 *Pi*). The substance constituting the thickened portions of this wall was found to be cellulose or a cellulose-like substance. These 3 layers of thickened cells doubtless serve to protect the seed. The remaining tissue of the ovarian wall, aside from the vascular bundles, consists of thin-walled parenchymatous cells.

Just within the layers of thickened cells the primary vascular bundles of the carpel are found. There are three of these, one growing up from the base of the ovary along the middle of each flat face (figs. 11 *VB*, 21 *VB-ov*, 42). These bundles are laid down very early in carpellary development and extend for a considerable distance into the tissue of the bract. These bundles extend upward in the wall of the ovary to the level of the chalaza of the ovule, where each bundle divides into two. One of each of these turns inward and bends downward to enter the base of the ovule (figs. 21, 22). From one or perhaps sometimes from each of the three nucellar branches there arises a strand which passes upward to form the single vascular bundle of the style. The second branch of each of the primary carpellary bundles turns outward, away from the nucellus, and passes out from the tissue of the carpel into the perianth through the zone of attachment near the top of the ovule, where the tissue of the carpel and perianth are continuous (figs. 14, 21, 22). Immediately after entering the perianth this perianth branch of the carpellary bundle turns upward to

end in the terminal lobe of the perianth. At either side as it turns upward this branch is joined by a branch from the perianth bundle itself (fig. 21 *x*). The presence in the wall of the ovary of 3 distinct bundles lends strong support to the view that the ovary is made up of 3 carpels, although no evidence of separate lobes could be distinguished at the upgrowing margin of the young ovary.

OVULE.—The nucellus of the ovule is initiated by the inward and downward growth, on the side opposite the bract, of the subepidermal cells of the wall of the ovary which border on the ovarian cavity (figs. 14, 18). The integuments arise soon after the carpel has closed in above the ovule to form the style and stigma. The inner integument starts as a ringlike outgrowth from the sides of the ovule near its middle. This is due chiefly to the activity of its epidermal cells (fig. 26). Soon after the inner integument appears, a second outgrowth slightly anterior to its base leads to the development of the outer integument (figs. 26 *OIn*, 28). These ringlike outgrowths continue growing downward around the ovule, that is, toward the micropyle, as it elongates. By the time the tapetal cell has divided, producing the 4 or 5 layers of cells constituting the tapetum, and the megaspore mother cell has come to occupy a central position within the nucellus, the inner integument has completely closed together above the nucellus to form the micropyle (fig. 28). The inner integument is longer than the outer from the outset of its development. At the time the embryo sac is ripe, the integuments extend considerably beyond the nucellus into the ovarian cavity, and each is 3 cells in thickness. From this time on neither the integuments nor nucellus change appreciably in appearance until the developing endosperm crushes them against the wall of the ovary where the integuments form the seed coat (fig. 42).

The 3 vascular bundles of the ovary can be distinguished entering the base of the nucellus almost immediately after its initiation (fig. 11, lower flower). The vascular bundle (indicated by dotted line in fig. 14) consists of cells which are elongating actively toward the nucellus, but the walls of which have not yet begun to thicken. When the embryo sac is mature the nucellus

consists of 7 or 8 layers of cells between the walls of the embryo sac and the outer surface of the nucellus.

The nucellus in the mature seed has been completely absorbed by the swelling endosperm, and there is left only the crushed remains of its cells against the two integuments, the inner of which is considerably thickened in the micropylar region (fig. 42).

Embryo sac

ARCHESPORIUM AND TAPETUM.—Of the group of hypodermal cells seen in the young ovule at the beginning of its development, two axial cells become distinguishable, owing to their larger size and larger nuclei, at the time the outer integument is clearly evident (fig. 26). These two cells are the parietal or tapetal cell above and the definitive archesporial or megaspore mother cell toward the chalaza (fig. 26). The tapetal cell divides early to form the 4 or 5 layers of parietal tissue found in the young seed above the sac (figs. 27–29). The cells of the tapetum thus formed do not change appreciably until crushed by the growth of the endosperm after the formation of the embryo.

The definitive archesporial cell is now considerably elongated and contains a large nucleus (fig. 28). This divides in the usual way to form 3 or more (frequently 4) potential megaspores, as is evident from the condition seen in figs. 29 and 30. The chalazal megaspore of the group is the one which becomes the functional megaspore and develops into the embryo sac, while the 2 or 3 micropylar ones degenerate and are later absorbed (figs. 29–31). The functional megaspore increases steadily in size during the degeneration of the non-functional megaspores, and then evidently begins to divide in the usual way, giving rise to 2, 4, and 8-nucleate stages. The first division of the mother cell nucleus was not observed, but the position within the embryo sac of the nuclei of its second division indicates a definite polarity after the first division, which is evidently maintained on through the subsequent divisions (figs. 31, 32).

The mature embryo sac is of the 7-nucleate type common to most angiosperms (fig. 33). The embryo sac continues to increase greatly in size, and the egg apparatus and endosperm nucleus are

seen to occupy typical positions within it (fig. 34). The endosperm nucleus at the time of its formation by the fusion of its polar nuclei and for some time thereafter contains two nucleoli. Later, however, but a single large nucleolus is visible (figs. 34, 35).

FERTILIZATION.—The writer has not investigated the details of the germination of the pollen grain upon the stigma, nor of the penetration of the pollen tube through the style into the ovarian cavity. From what was observed, it appears that the pollen tube enters the embryo sac in the usual way, displacing and disorganizing the synergids (figs. 35, 37).

The relative time of fusion of male nucleus with the egg was not determined with certainty, but it probably remains for some time in close proximity to the egg without fusion, during which time the endosperm nucleus proceeds to divide actively. The first division of the endosperm nucleus is followed immediately by the formation of a cell wall (fig. 36). Hence it may be said that the endosperm is "cellular from the outset of its development" (JOHNSON 9). The egg in *Hedyosmum nutans*, even when the endosperm has reached the 4-celled stage, is still undivided and uninucleolate (fig. 37). In *Hedyosmum arborescens* the fertilized egg is still undivided and binucleolate when the endosperm has reached the 10–12-celled stage (fig. 38). In the earliest stages of the 2-celled embryo seen, the number of endosperm cells is approximately 100. The endosperm continues to divide by longitudinal and transverse walls and rapidly encroaches on the substance of the nucellus (fig. 41). In the mature seed the endosperm has completely crushed and absorbed all but one layer of the nucellar tissue, so that the endosperm lies practically in contact with the inner integument (fig. 43). The antipodals persist unchanged for some time, but as the division of the endosperm continues they begin to degenerate, and finally disappear altogether. The embryo develops into a slightly elongated or oval mass of cells with a poorly developed suspensor (fig. 43).

The chief protection of the seed, except near the micropylar end, where the inner integument is considerably thickened, is afforded by the peculiar thick-walled, pitted cells of the carpel

(figs. 22, 23, 42). The ripe seed is somewhat oval in side view, but sharply triangular in cross-section.

Germination of seed

Observations of the germination of the seed were made in this laboratory by JOHNSON. It is of the type commonly occurring in endosperm-containing angiosperms. The small globular embryo differentiates gradually, and as the hypocotyl grows out of the nucellar region the cotyledons are extended on into the endosperm toward the chalaza. Here they remain until the stored food material is practically exhausted (fig. 44). Later they become freed from the endosperm, expand, and assume active photosynthetic functions.

Discussion

Certain peculiarities in the structure and development of the reproductive organs of *Hedyosmum nutans* which have been described suggest certain conclusions which may be drawn as to its phylogenetic origin. Correlated with these is the question of the comparative primitiveness of this plant in relation to other members of its family and order, and to other angiosperms.

CAMPBELL (3) has studied *Peperomia pellucida*, and JOHNSON has made detailed studies of this and 4 other species of the Piperaceae, as well as of representative genera of the other 3 families of the Piperales. Both investigators have called attention to certain facts bearing also on the relationship of the Chloranthaceae. It is on the basis of the views of these earlier investigators that the writer attempts to interpret the peculiarities occurring in *Hedyosmum*.

An interesting feature of the reproductive structures of this plant is the difference in character of the staminate and pistillate inflorescences. Whereas the staminate flowers occur in long-stalked ovoid catkins (see ENGLER and PRANTL **7**, fig. 13), the pistillate flowers, on the contrary, occur in sparsely flowered panicles. The occurrence of these two distinct types of inflorescence, along with the differentiation of the flowers into strictly staminate and pistillate ones, is to be noted in contrasting the

plants of this family with the other 3 families of the Piperales. In all three of the latter, the Saururaceae, Piperaceae, and Lacistemaceae, the flowers are hermaphrodite (except in *Piper Betel* L. var. *monoicum* C.DC. according to JOHNSON **10,** p. 716) and are arranged in distinct catkins. The occurrence of unisexual flowers is generally regarded as a mark of specialization within the family or order, and their presence in *Hedyosmum* indicates that this is one of the more specialized Piperales, rather than a very primitive one.

Again, the perianth of *Hedyosmum* is not of a primitive type. In fact it is a very unusual one. In so far as the writer has reviewed the literature on angiosperms in general, and the Piperales in particular, he has not discovered any type of floral envelope which is at all closely similar to it in its origin or mature structure.

CLARKE, who was the first to describe this perianth and to note its pores, does not discuss its origin, but refers to it as a calyx. SOLMS mentions a structure which he denotes as a perigon trifid at the apex. BAILLON says the apex of the ovary bears 3 short, thick, rounded wings whose morphological value is still uncertain. EICHLER speaks of a 3-lobed half or quite superior perigon. BENTHAM and HOOKER mention a tube of the perianth as grown to the ovary.

The endosperm, which is cellular from the outset of its development, finds its parallel in this respect in *Heckeria* and *Peperomia*, but differs from that of *Piper* (JOHNSON **8, 10**), the only other genus of Piperaceae whose development is known. Elsewhere among dicotyledons the type of endosperm formation found in *Hedyosmum* occurs only in highly specialized forms (JOHNSON **9**). The families in which this is found are placed by ENGLER in the higher orders of the Archichlamydeae and Metachlamydeae. It would seem, therefore, that the conclusion reached by HOFMEISTER, STRASBURGER, and HEGELMAIER, that the structure and development of the gametophyte of angiosperms are not a satisfactory index of broader genetic relationships, finds support here in this peculiarity of the structure and development of the perianth and endosperm of *Hedyosmum*.

Other peculiarities of endosperm development, however, such as the complete replacement of nucellar tissue by endosperm, occur more constantly throughout large groups than such features as a single type of tapetum or embryo sac, or of a particular number of potential megaspores. These peculiarities, therefore, may have considerable weight in determining such broader relationships.

Summary

1. The staminate flowers usually occur in long-stalked ovoid catkins which arise in a pair at the base of the cymelike female inflorescence. Each stamen possesses 4 distinct microsporangia.

2. The carpellate flowers occur in sparsely flowered panicles. They have a single pistil, although there is some evidence that 3 carpels enter into the formation of this pistil (**5**). The perianth of the female flower is initiated before the carpel. It is connected with the surface of the ovary by means of a narrow longitudinal band of tissue which extends along each of its 3 corners; by a similar narrow zone about its base; and by another zone of attachment around the ovary in the apical region just below the base of the style. The perianth persists in the mature fruit and probably constitutes an added protection to the seed.

3. The ovary is 1-celled. Its wall is composed of 15 layers of cells of which the epidermis and the next 3 layers within it are small and but slightly thickened. The cell walls of the fifth, sixth, and seventh layers adjoining the 4 just mentioned are at first unevenly thickened, then, during the ripening of the seed, the entire cavity of each of the cells becomes filled. The inner layer of cells of the ovarian wall next the seed is also considerably thickened in the ripe seed. These 3 thickened layers form the chief protection of the ripe seed.

4. The ovule is pendulous and orthotropic. It bears 2 integuments, the inner being the longer from the outset of development. These are quite thick in the mature seed around the micropylar region, but elsewhere they are unthickened and are scarcely discoverable in the mature seed.

5. A primary archesporium arises from a hypodermal cell of the nucellus, which by dividing produces a tapetal cell and a

definitive archesporial cell. The latter divides into three (usually four) potential megaspores. The lower or chalazal one of these is the functional one, the remaining three degenerate and are absorbed. The mature 7-nucleate embryo sac is of the type most characteristic of angiosperms.

6. The endosperm nucleus, embodying two polar nuclei and possibly a male nucleus, begins to divide before the oospore. Its first division is immediately followed by a transverse wall which divides the embryo sac into upper and lower cells. Each of these two cells continues to divide repeatedly, thus forming the thousands of endosperm cells that completely fill the mature seed except for the embryo. The several layers immediately surrounding the embryo are at this time devoid of starch.

7. The fertilized egg begins to divide only after 15–20 endosperm cells have been formed. The synergids and antipodals degenerate by the time of the first division of the oospore.

8. The ripe seed consists of a globular mass of cells with a poorly developed suspensor. The seed coat is not developed appreciably. Its function is obviously performed by the wall of the ovary.

9. At germination the small embryo is for a long time inclosed and nourished by the swelling endosperm. The cotyledons remain in the endosperm until nearly all its starch is exhausted. They are then withdrawn and assume an active photosynthetic function.

JOHNS HOPKINS UNIVERSITY
BALTIMORE, MD.

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EXPLANATION OF PLATES XXXIV–XXXVI

All figures are camera drawings from microtome sections except figs. 9, 10, 24, and 25. Abbreviations used: *Ant*, antipodals; *Br*, floral bract; *EdN*, endosperm nucleus; *Em*, embryo; *Esp*, endosperm; *Fl*, flower; *In*, integument; *IIn*, inner integument; *MN*, male nucleus; *MMC*, megaspore mother cell; *OC*, oil containing cell; *OIn*, outer integument; *Osp*, oospore; *OvC*, ovarian cavity; *PC*, perianth cavity; *Pe*, perianth; *Pi*, pits; *Po*, pore; *PT*, pollen tube; *Sg*, synergid; *SyC*, stylar canal; *Tp*, tapetal cell and tapetum; *VB*, vascular bundle; *VB-ov*, *VB-nc*, *VB-pe*, *VB-sy*, vascular bundles of ovary, nucellus, perianth, and style; *WO*, wall of ovary.

FIG. 1.—Transverse section of stamen showing 3-layered wall, tapetum, and young pollen mother cells; ×110.

FIG. 2.—Detailed drawing of archesporium of one microsporangium with tapetum, etc., shown in fig. 1; ×650.

FIG. 3.—Tetrad of young pollen grains; ×1230.

FIG. 4.—Transverse section of nearly mature anther; ×110.

FIG. 5.—Section of mature pollen grain; ×1100.

FIG. 6.—Polar view of wall of mature pollen grain; ×1100.

FIG. 7.—Longitudinal section of young female flower and bracts; ×55.

FIG. 8.—Similar section of two flowers, upper slightly older than one shown in fig. 7; ×55.

FIG. 9.—Surface view on one side of a young female flower; ×55.

FIG. 10.—Surface view of one side of older female flower; ×55.

FIG. 11.—Transverse section of female inflorescence showing various early stages in carpellary development; ×50.

FIG. 12.—Longitudinal section of female inflorescence; ×50.

FIG. 13.—Similar section of very young flower showing initiation of ovarian cavity; ×550.

FIG. 14.—Similar section at later stage showing initiation of style and stigma; ×250.

FIGS. 15–20.—Successive transverse sections (numbered from base to apex) of single young female flower and subtending bract; ×55.

FIG. 21.—Diagram of wax model of vascular bundle system of perianth and ovary.

FIG. 22.—Longitudinal section of flower at time of ripe embryo sac; ×60.

FIG. 23.—Tangential section of one of thickened pitted cells of ovarian wall; ×70.

FIG. 24.—Surface view of young inflorescence showing bracts and two young flowers; ×20.

FIG. 25.—Surface view of ripe fruit; ×20.

FIG. 26.—Nearly longitudinal section of young ovule showing tapetal and definitive archesporial cells; ×650.

FIG. 27.—Similar section showing inner and outer integuments, origin of tapetum, and increase in size of megaspore mother cell; ×650.

FIG. 28.—Similar section of ovule at still later stage showing inner integument closed above nucellus to form micropyle; ×650.

FIG. 29.—Longitudinal section of nucellus showing young embryo sac with 2 degenerating megaspores above it; ×600.

FIG. 30.—Similar section at later stage showing embryo sac and 3 degenerating megaspores; ×600.

FIG. 31.—Similar section of older nucellus showing polarity of 4-nucleate embryo sac; ×600.

FIG. 32.—Longitudinal section of embryo sac showing mitosis in division of 4 nuclei like those shown in fig. 31; ×1100.

FIG. 33.—Similar section of a mature 7-nucleate embryo sac; ×570.

FIG. 34.—Similar section of micropylar half of mature embryo sac showing typical position of egg apparatus and uninucleolate endosperm nucleus; ×570.

FIG. 35.—Similar section of embryo sac after pollen tube has entered; ×570.

FIG. 36.—Longitudinal section of embryo sac showing first two endosperm cells; ×600.

FIG. 37.—Similar section showing further division of endosperm and antipodals still persistent at base of embryo sac; ×600.

FIG. 38.—Similar section of *H. arborescens* at slightly later stage than shown in fig. 37; ×600.

FIG. 39.—Similar section of micropylar portion of embryo sac at 3-celled stage of embryo; ×500.

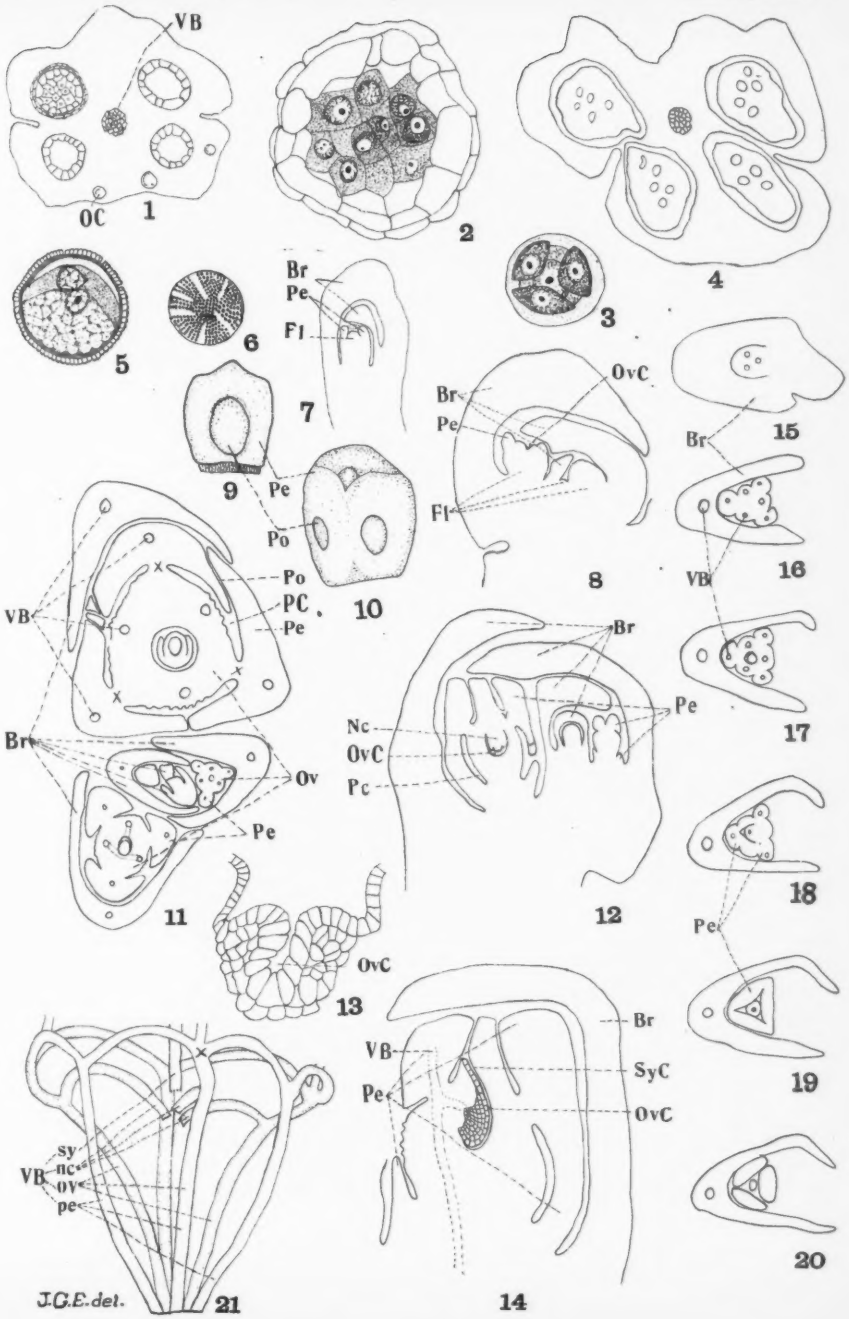
FIG. 40.—Drawing of similar section at later stage showing embryo; ×500.

FIG. 41.—Longitudinal section of micropylar end of nucellus and embryo sac showing character of endosperm around embryo; ×650.

FIG. 42.—Longitudinal section of nearly mature seed showing embryo, endosperm, remains of nucellus, and surrounding wall of ovary with protective cells and still persistent perianth; ×60.

FIG. 43.—Similar section of nearly ripe seed showing embryo, endosperm, and one integument at micropylar end; ×125.

FIG. 44.—Longitudinal section of germinating seed removed from fruit showing absorbent cotyledons still imbedded in endosperm; ×60.



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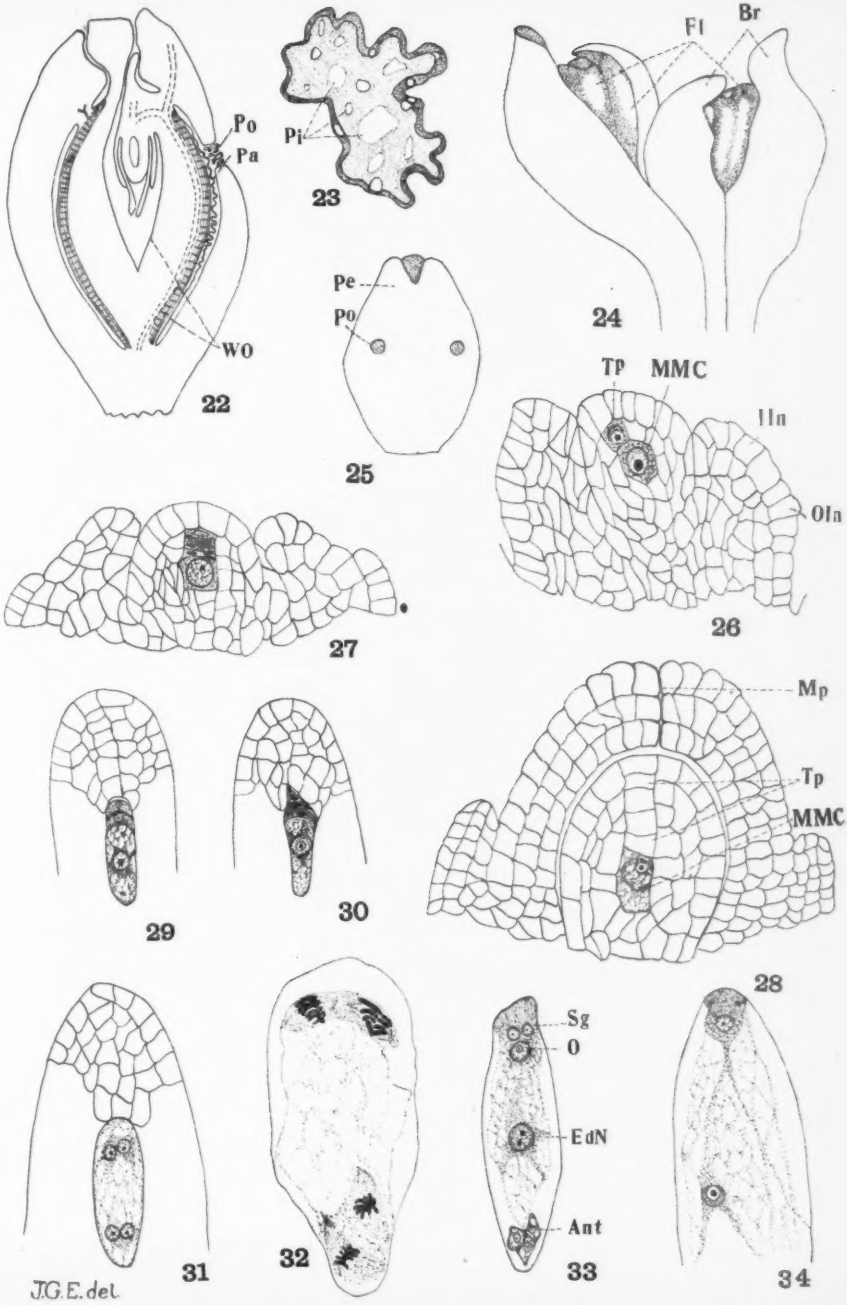

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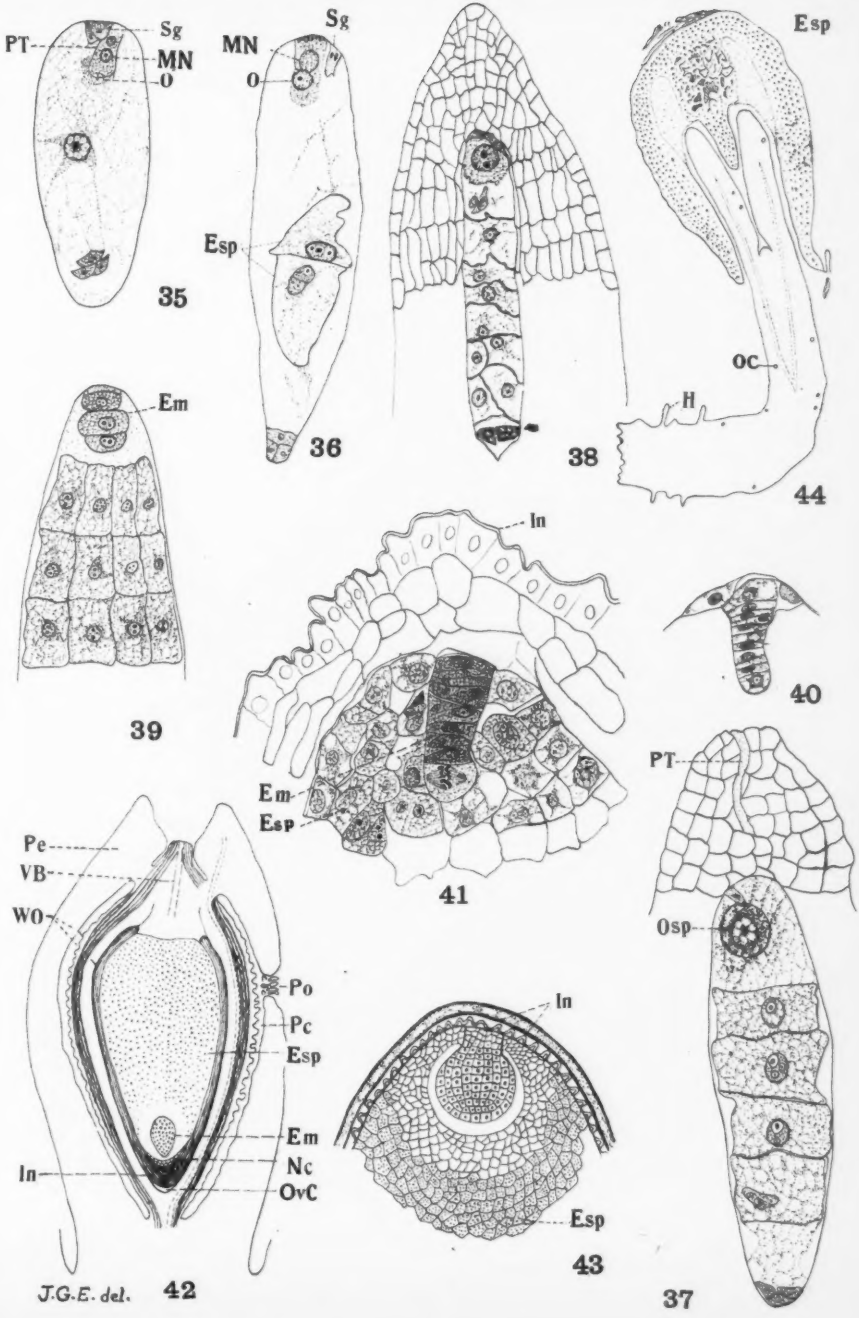
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SOME CHARACTERS OF XYLEM TISSUE IN CYCADS

H. B. SIFTON

(WITH PLATES XXXVII, XXXVIII AND ONE FIGURE)

The detailed investigation of certain anatomical features of the Cycads has been undertaken in the hope of throwing light on the origin of the more specialized structures occurring in the higher Gymnosperms. In recent years considerable work has been devoted to determining the details of anatomical structure in the Conifers. These details have been given much prominence as evidences of the inter-relationships of the various groups. A lack of knowledge of the ancestry of the structures themselves, however, has minimized their value as criteria in phylogenetic investigations. This knowledge can be supplied only by a study of primitive forms.

Pitting

The shape and arrangement of bordered pits in the woody tissue have long been regarded as valuable phylogenetic data. It is largely owing to these features that the Araucarians have been supposed to be closely related to Cordaitean forms, and many botanists still hold this view, notwithstanding the arguments advanced by JEFFREY (**6**) and SEWARD (**9**) in favor of different lines of ancestry for the family. In 1907 GOTHAN (**4**) worked out a phylogenetic line of development of bordered pitting, considering the most primitive type to be hexagonal and crowded over the whole tracheid wall. According to his theory, the pitting next became eliminated from the tangential walls, but still covered the radial as before. A further elimination resulted first in small isolated groups of pits, then in the uniseriate flattened condition, and finally in the scattered arrangement, where the pits occur singly on the tracheid wall. This series of eliminations took place on the middle part of the wall of the tracheid, the crowded arrangement being retained on the ends to facilitate vertical movement of the sap. GOTHAN found his types of arrangement combined in a fossil plant, but in no living form.

JEFFREY is more conservative than many others in his estimate of the significance of pitting, but considers that it is of distinct value in classification when its character in all parts of the plant is considered. On this ground, in his work on the Araucarineae, he accepts the presence of opposition pitting in the cone axis, and scattered pits in the seedlings, as denoting descent from an Abietineous type. He has neglected the character of the pitting in primitive forms such as the Cycads, however, and his interpretation is not in harmony with the facts which these forms disclose. This was done, notwithstanding the fact that as early as 1840 DON (**2**) recognized the value in phylogeny of the study of Cycads. He carefully worked over the character of tracheids by such methods as were in vogue at that time, and agreed with MEYENS, a still earlier investigator, that the spiral, scalariform, reticulate, and border-pitted types could be referred to a common origin. The importance of these transitions was emphasized also by PENHALLOW (**7**) in 1907 as affording valuable data on the ancestral character of the bordered pit of the higher forms. In 1919 BAILEY (**1**) argued that opposite pitting is formed by the breaking up of bordered scalariforms, and that the alternate type was formed from this by a "staggering" of the rows of pits.

In this paper certain features of the primary wood of the Cycads will be considered first. Fig. 1 is a longitudinal section of the petiole of *Cycas revoluta*, showing the tangential walls of the tracheids in the neighborhood of the protoxylem. The tracheid *a* shows the characteristic spiral and scalariform structure of the protoxylem. In transverse section (not figured) the scalariform bars are seen to arch over the intervening spaces so as to form very narrow borders. On the cell *b* the scalariforms are more closely approximated, and through the slits may be seen shorter pores, belonging to the adjacent wall of the next tracheid. The tracheid *c* also shows this clearly. In the other two tracheids typical bordered pits are present. The type of scalariform from which such bordered pits are formed is shown in fig. 2. It is a scalariform similar to that formerly described, except that the borders are wider. Fig. 3, another section from a *Cycas* petiole, indicates transitions in the formation of ordinary bordered pits from this

type of scalariform. Below the center of the figure is a scalariform reaching from side to side. Its border shows constrictions at two points, evidently the beginning of a division into three bordered pits. In the portions of the tracheid above and below, complete divisions and other incomplete ones are in evidence. The name "fusion pits," which has been applied to similar appearances, is evidently a misnomer in this case. They plainly represent phases in the breaking up of the ancestral scalariform rather than the union of two of the more specialized bordered pits. The small pits on tracheids *a* and *b* of fig. 1 in all probability are formed from the narrow bordered scalariforms in a similar manner.

Figure 4 is a much lower magnification of a longitudinal radial section of the fossil stem of *Lyginodendron Oldhamium*, acknowledged to be one of the most ancient of the seed plants. This form had attained in the secondary wood of its stem the condition represented in GOTHAN'S second type, the pits being practically eliminated from the tangential walls (cf. SCOTT **8**), but crowding the radial walls from end to end of the tracheid. Wherever the cell wall is present in the figure it is seen to be completely covered with the type of pitting known as recticulate. A careful examination of the pits shows them to be of the same type as those in fig. 1, which had their origin in the narrow bordered scalariforms.

The stem of *Cordaites* (fig. 5) has pits which, like those of the Cycads, have probably originated from the cutting up of wide bordered scalariforms, a condition quite in keeping with the general higher type of wood structure exhibited in the Cordaitean forms.

Further evidence of the origin of the bordered pit from the scalariform is found in the secondary wood of certain of the Cycads. A type of fusion pit which recalls the condition in the narrow bordered scalariforms of fig. 1 is shown in fig. 10, which is a radial section of the stem of *Dioon spinulosum*. The three pits nearest the top are of the short, slightly flattened type quite common in these forms. The next three are more elongated. All show the characteristic cross pores of adjacent elements. The seventh of the series is a pit of the second fusion type. It appears like two pits, each with a short pore, with a common long pore crossing both.

On one tracheid wall (in this case the one beneath) two separate pits have formed, each with its own pore; while on the adjacent wall of the next tracheid, one large, somewhat scalariform pit has been retained. Just below this comes a pair of completely separate pits, from their shape and approximation evidently formed by the division of what potentially was a single long one. Then come two more pits of the fusion type, after which the regular type of pitting is resumed. Such examples are often found at the ends of tracheids, where, as noted later, there are other primitive characters.

The multiseriate condition is the most common arrangement of pits in Cycads. In some cases the pits are so closely approximate as to appear slightly flattened. This is the typical condition in the Cordaiteae as described by SCOTT, and has generally been considered the most primitive bordered condition, although more specialized than the reticulate type. The outline of the pits in the Cycads, however, is more often curved.

In the Cycads the scattered type of pitting is also found, originating by the elimination of pits from portions of the tracheid. Fig. 6 from a radial section of a *Dioon spinulosum* stem shows this feature. In the lower part of the right hand tracheid we have biseriate pitting covering the radial wall, with here and there a pit obliterated. The position of the vanished pits is indicated by lighter areas caused by the thinning of the primary wall. These are the primordial pits of Sanio, which have formed as if bordered pits were to be located over them as usual. Farther toward the top is a single row of somewhat flattened pits, an arrangement common in the Araucarians. Still farther up some of the pits have become smaller, while others have been eliminated entirely, thinning of the primary wall being visible here and there. This scattered pitting is seen also in other tracheids of the figure, the primary pit areas being very evident, especially just above and below the center of the second tracheid from the left. At the center is an interesting small bordered pit surrounded by a slightly larger area, probably the boundary of the primordial pit. This seems like a case of partial elimination. The irregular obliteration of the pits has left in some places isolated groups of pits like those

referred to by GOTHAN. A further eccentricity of the elimination is illustrated in the tracheid to the right of fig. 11. The lighter colored pits have typical bilateral borders, while the obscure ones are unilateral, the corresponding pit on the overlying tracheid not having been formed.

The arrangement of pits, opposite or alternate, deserves notice. In fig. 6 two or three pits occur in a horizontal row. In some cases as many as four such pits have been found. In the more common condition, not figured, the pits are regularly alternate, sometimes round, and sometimes flattened by mutual contact. Just as the scalariforms of one tracheid are horizontal while those of the next are inclined, and either straight or curved and irregular (fig. 1), so on one tracheid is found the opposite arrangement of pits, and adjacent to it the alternate. There seems no reason for believing that alternate pitting is formed by any disarrangement of rows of round bordered pits. More specialized plants have one or other of these types predominating; for example, the alternate and flattened arrangement in Araucarineae, and the scattered, grouped, and opposite in the Abietineae. The presence of all these conditions in mature Cycad wood, as well as in the ancient fossil form described by GOTHAN, modifies to a great extent their phylogenetic significance in higher forms. It strengthens BAILEY'S statement that the presence of opposite pitting as well as alternate in the cone axes of Araucarians cannot properly be used as an argument for their descent from the Abietineae, and neither is the same condition in primitive parts of pines an evidence of descent from an Araucarian type. It would seem that if these facts have any significance in phylogeny, they indicate that both pines and Araucarians are descended from lower forms which contained both these arrangements.

It may be stated in passing that both opposite and alternate arrangements of pitting occur in the Cordaiteae, the alternate, however, being greatly predominant. Instances of opposition pitting in Cordaites may be seen in fig. 5, in the upper part of the right hand pitted tracheid. There is an example also near the lower end of the second tracheid to the left of it. Instances of GOTHAN'S grouped arrangement are also present, especially in the

lower central part of the figure. The uniseriate and scattered arrangements occur, being formed probably by the suppression of pits, as in the Cycads. The only evidence of this in the fossil section, however, is the decreased size of many of the pits in the region of elimination. A careful examination of fig. 5 will make this point clear.

It will be seen from the foregoing account that the Cycads, besides giving indication of the mode of formation of the bordered pits from the scalariform type, afford valuable data on the interpretation of the arrangement of pits. The elimination in these low

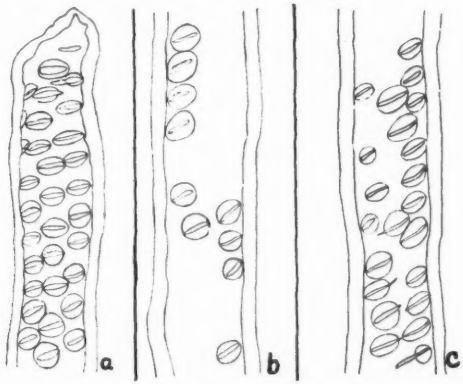

FIG. 1.—*Cycas revoluta:* radial views of different regions of tracheid from stem; *a*, end of tracheid; *b*, normal pitting at contact of two tracheids; *c*, pitting in contact with medullary ray.

forms shows no indication of following a definite law, but proceeds promiscuously, giving rise to all the various types of pitting. It is practically restricted, however, to the middle part of the tracheid, the terminal portions and those parts in contact with the ray cells remaining multiseriate.

The terminal and ray pitting of the tracheids has always remained primitive in another respect. This is indicated in text-fig. 1, which is from a tracheid of the stem of *Cycas revoluta*. In this figure *a* represents the terminal pitting, *b* the ordinary pitting, and *c* the ray pitting of the same tracheid. The pits and their pores are longer in *a* and *c* than in *b*. The pores often extend

beyond the borders in *a* and *c*, and thus recall the condition where pits are forming on scalariform elements (fig. 3). Many of the pores were measured for definite comparison, and the tracheid figured does not exaggerate the difference in pit and pore lengths. Ray pitting on *Dioon spinulosum* secondary stem wood is shown in fig. 9. Here also the multiseriate pitting is present, accompanied by the elongated pore. The left hand tracheid of fig. 11 shows the same type of pore, where a vertical parenchyma cell is in contact with a tracheid. Evidently the primitive type of pore occurs wherever a tracheid is in contact with any type of parenchyma cell. Similar primitive features have been recorded by THOMSON (11) in Araucarian ray pitting.

Tertiary thickenings are common on the tracheid walls, taking the form of spirals or scalariform bars with long shallow pits between. They occur whether bordered pits are present or not, and often traverse the region of the border itself, but have never been observed to cross the pores. PENHALLOW regarded such thickenings as relics of the ancestral manner of deposition of the cell wall, a view which is strengthened by their presence in these low forms.

Bars or rims of Sanio

Considerable importance was attached for some years to the presence or absence of "bars" or "rims" of Sanio. Miss GERRY (3) in 1910 showed them to be present in all families of the Conifers except the Araucarians, and made this a distinguishing feature between both fossil and living Araucarians and other coniferous forms. JEFFREY (6) and THOMSON (11), however, in practically simultaneous publications described bars from the transitional region of the pitting in the cone axis of an Araucarian. This JEFFREY interpreted as evidence of the derivation of Araucarians from the Abietineae. He recognized that this evidence would be invalid if all primitive types of pitting had bars of Sanio, and looked for them in primitive regions of *Cycas* but failed to find them. Their presence here was described later by the writer (10), and invalidates his conclusions. JEFFREY'S misstatement has no doubt been responsible for the exaggeration of the importance of

the structure, and probably led to the rejection of all other criteria, making the bar "an infallible test for tribal affinities" (HOLDEN 5), both in fossil and living Conifers.

In 1919 BAILEY (1) studied the origin and development of bars of Sanio, and concluded that those in transition regions are merely normal middle lamellae left between thinned pit areas in the primary wall. He states that when the pits are opposite the bars go smoothly from side to side of the tracheid, because the pits are formed on one primary scalariform pit area, and the bar is the thickening of the lamellae between this area and the next. This theory, however, will not explain the bars figured in the writer's paper of 1915. Those shown in *Araucaria* are connected with pits in regular horizontal rows, but still fork, following round the circumference of each pit, so as to leave clear diamond-shaped areas bounded by opposing forks. These small areas cannot be considered to be other primary pits. The same fact holds for the bars in the *Cycas* petiole, which fork, and are even split into two separate rims in some cases, although the pits are not far enough apart to make it possible to attribute the thin space to another primary pit area.

Bars of Sanio have now been found in other portions of Cycads than the transitional primary xylem. Figs. 6 and 7 illustrate them in the stem wood of *Dioon spinulosum*. These bars often extend beyond the margins of the pits with which they are in contact, as in the middle tracheid of fig. 7 near the bottom, and so are of a higher type than those figured in the former paper. They are still much more primitive than those of the Abietineae, however, lying in close contact with the pits, whenever such are present. Fig. 6 shows the ordinary type of bar in this plant. Between the pits of the single row on the right are bars of the regular Araucarian type. Their length is not greater than the borders to which they cling, and they spread slightly at the ends. The pitting of this tracheid is conspicuously of the opposite type, so that if BAILEY'S theory of the origin of the bars is entirely correct, they should in this case pass beyond the pits to the limit of the tracheids. In the two left-hand tracheids of fig. 6 is shown a condition which is quite common, namely, the presence of these bars in connection

with primordial pits from which the secondary pitting has been eliminated.

Trabeculae

These spool-shaped bars, extending in radial series across the lumens of adjacent tracheids, have received considerable notice in literature, owing to the confusion which arose in some cases between them and bars of Sanio. They have not before been figured in the Cycads, but are present, as shown in fig. 8, a radial section of *Dioon spinulosum* stem. They contain a core or axis composed of a substance which stains in the same way as the middle lamella of the cell. This core pierces the tangential secondary walls of the tracheid and joins up with the middle lamella. These structures are present in higher forms, but their significance is not known. Since they connect with the primary wall, they must have been laid down before the beginning of secondary thickening.

Summary

1. A study of the primary and secondary wood of Cycads indicates the development of reticulate, alternate, and opposite pitting directly from scalariform types.

2. The grouped, uniseriate, and scattered pitting characteristic of higher forms is shown to be formed by the elimination of pits. In low forms, of which the Cycads are a type, this elimination proceeded without apparent order, forming all types of grouping indiscriminately.

3. Similar arrangements of pits occur in Cordaites, although its type has become more fixed than is the case in the Cycads.

4. The Cycads, like the Araucarians, have more primitive types of pitting at the ends of tracheids and where they come in contact with parenchyma.

5. The xylem of certain of the Cycads quite commonly exhibits spiral tertiary thickenings.

6. Bars of Sanio of the Araucarian type are found in both primary and secondary Cycad wood. An elongated type of bar is also present. The Araucarian type is considered the most primitive in living seed plants. No explanation of its origin is offered by BAILEY'S theory.

7. Trabeculae are present.

This work was done with the advice of Professor R. B. THOMSON. I am indebted to him, not only for advice and encouragement, but also for the supply of materials necessary for the work.

UNIVERSITY OF TORONTO

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DESCRIPTION OF PLATES XXXVII, XXXVIII

PLATE XXXVII

FIG. 1.—*Cycas revoluta:* petiole; tangential section of primary wood; ×225.

FIG. 2.—*Zamia integrifolia:* petiole; pitting of primary wood; ×445.

FIG. 3.—*Cycas revoluta:* petiole; primary wood, showing transitional pitting; ×445.

FIG. 4.—*Lygenodendron Oldhamium:* radial section of secondary wood of stem; ×100.

FIG. 5.—*Cordaites* sp.: radial section of secondary wood of stem; ×225.

PLATE XXXVIII

FIG. 6.—*Dioon spinulosum:* radial section of secondary wood of stem, showing pit arrangement and bars of Sanio; ×225.

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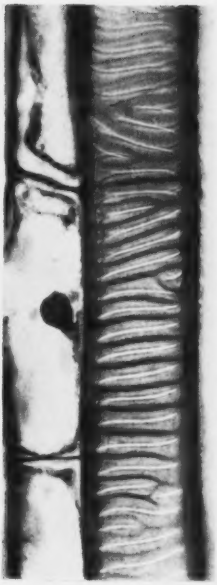

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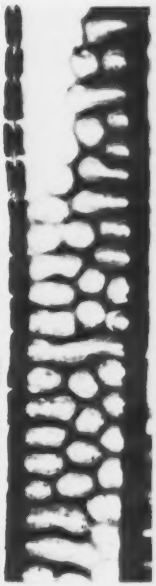

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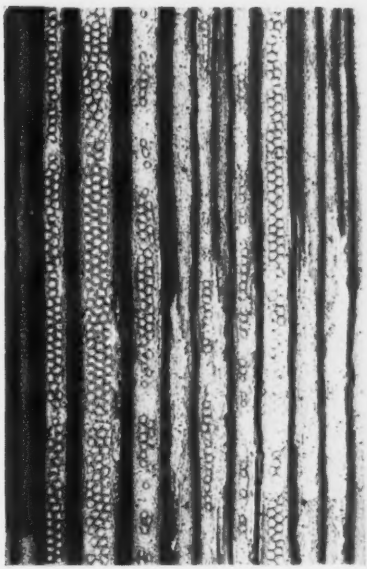

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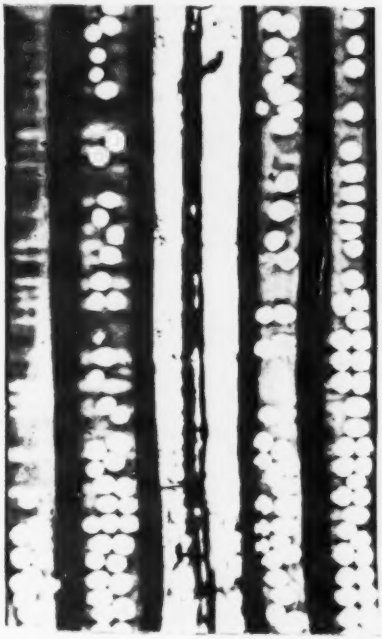
SIFTON on CYCADS



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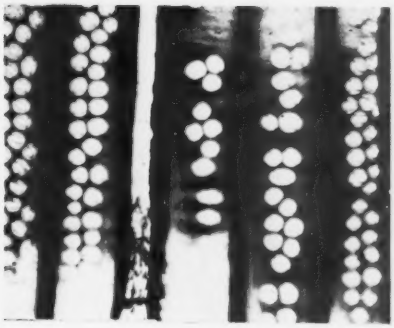

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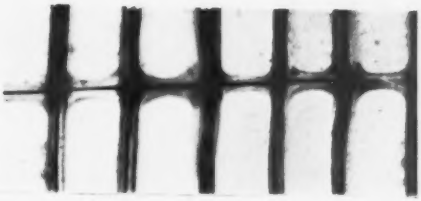

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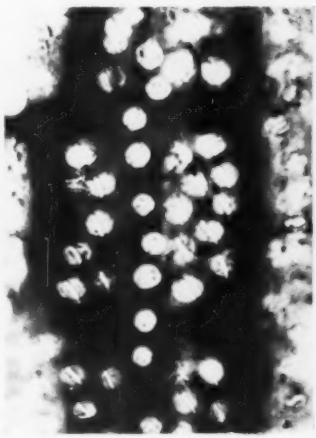

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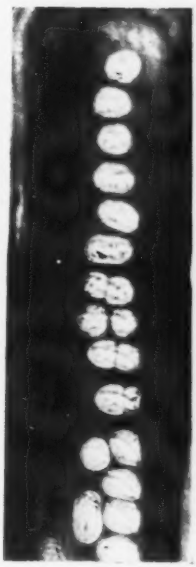

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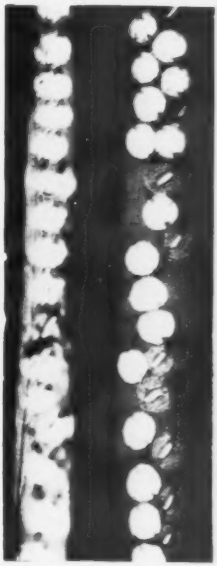

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SIFTON on CYCADS

FIG. 7.—*Dioon spinulosum:* radial section of secondary wood of stem, showing pit arrangement and bars of Sanio; ×225.

FIG. 8.—*Dioon spinulosum:* radial section of secondary wood of stem, showing trabeculae; ×225.

FIG. 9.—*Dioon spinulosum:* radial section of secondary wood of stem, showing pitting in contact with medullary ray; ×445.

FIG. 10.—*Dioon spinulosum:* radial section of secondary wood of stem, with fusion pits near end of tracheid; ×445.

FIG. 11.—*Dioon spinulosum:* radial section of secondary wood of stem, showing pitting in contact with wood parenchyma, and unilateral pits between two tracheids; ×445.

DEVELOPMENT OF EMBRYO OF GNETUM

HULDA I. HAINING

(WITH PLATES XXXIX–XLI AND ONE FIGURE)

In his paper on the morphology of *Gnetum*, THOMPSON (**6**) fully described the different phases in the reproduction of this genus, except the development of the embryo. The material for an investigation of the latter subject was turned over to me, and the results of my study are given in the following pages.

The species studied included *G. funiculare*, and *G.* sp. 15, *G.* sp. 29, and *G.* sp. 59 of the Buitenzorg Botanic Garden. Certain stages were also studied in *G. Gnemon*. The young stages and thicker parts of the embryo were cut in serial section. Preparations of the ripe red fruit and germinating seeds of *G.* sp. 15, *G.* sp. 29, and *G.* sp. 59 were made by dissecting out the embryos with their tortuous suspensors, which were then stained in Delafield's haematoxylin, extended, and mounted in balsam. On account of the widely branching character of the suspensors this method could not be used for *G. funiculare* and *G. Gnemon*, and it was necessary to make serial sections.

History

BOWER (**1**) gives the following account of the history of the work on the embryo of *Gnetum*, prior to his own study of the subject. In 1832 BLUME and GRIFFITH had observed in *G. scandens* and *G. latifolium* respectively that within a cavity in the endosperm a coiled mass of suspensors is formed bearing an embryo with two small cotyledons. HOOKER examined sections of *G. Gnemon* and found therein "tubular cells" which occasionally branch, permeating the apical part of the endosperm.

In 1882 BOWER published his account of the development of the embryo of *G. Gnemon*. He states that in the ripe seeds the suspensors bear no embryo, but have the appearance described by HOOKER. Although doubtful of the origin of the first cell of the embryo, formed after germination, he assumes that it is cut

off from the apex of the suspensor. Then he describes the formation of the multicellular embryo, in which an apical cell functions. No mention is made of long secondary suspensors, such as those observed in *G.* sp. 15 and *G.* sp. 59; but the description of the differentiation of the tissues in the embryonic body is in accord with that given later for these species. Reference is also made to seeds of an unknown species in which the suspensors form a coiled bundle in a cavity in the endosperm. This corresponds to the condition found in the ripe fruit of *G.* sp. 29; but it is stated that in the individual suspensors a single large nucleus appears toward the tip of the tube, and no reference is made to the peculiar cell which is present in *G.* sp. 29.

LOTSY examined *G. Gnemon* in 1899. He described the extensive suspensor-like elongation of the fertilized egg, the branching of this tube, and the cutting off of an embryo cell at the tip.

COULTER (**3**) reinvestigated the early stages in the development of the same species in 1908. He found that in the formation of the suspensor free nuclear division takes place, resulting in a few nuclei scattered along the suspensor, which are often separated by transverse walls. He describes a terminal embryo cell containing one of the free nuclei which continues to divide, accompanied by cleavage walls until a multicellular embryo is formed. This account differs from that given by BOWER in this respect, that the earlier writer described an apical cell as functioning, and no free nuclear division.

In 1916 THOMPSON (**6**) published observations on the embryo of several species of *Gnetum*. Differing from LOTSY'S account, he states that the fertilized egg of *G. Gnemon* divides into two cells, both of which develop into suspensors without transverse cleavage walls. In *G.* sp. 33 he describes a proembryo consisting of a small group of irregularly arranged cells, produced by division of the fertilized egg. Each of these cells then elongates, forming a tortuous suspensor which contains normally only one nucleus, although the possibility of the occurrence of several nuclei would not be excluded in all cases. Finally, he observed the suspensors of *G. moluccense* growing outside of the endosperm, between it and the nucellus. He states that the tips of the tubes enlarge within the

endosperm, and a group of cells is figured at the end of one of the suspensors,

Description

The early post-fertilization stages have not been satisfactorily determined by the writer, who has not observed the number of divisions of the fusion nucleus. The earliest stage which was recognized is that represented in the reconstruction of *G. funiculare* (fig. 1). This shows a large single-celled proembryo, from several points of which there have grown suspensors. Fig. 3 shows a later stage. While some of these tubes grow directly through the endosperm, others turn over the upper part of the embryo sac and make their way down the opposite side. These suspensors often form a tangle in the narrow part of the embryo sac. THOMPSON has stated that in *G. moluccense* suspensors are found between the endosperm and the nucellus. Several times the writer has observed the same condition in *G. funiculare*. In this species sometimes a tangle of tubes is observed in that position. At certain distances, cross-walls are formed in these suspensors, and branches grow into the endosperm (fig. 17). The ends of the suspensors in the endosperm have the usual dense protoplasm and nucleus.

In the earlier stages of the development of the embryos of *G.* sp. 15, *G.* sp. 29, and *G.* sp. 59 the endosperm becomes rather disorganized by the growth of tubes, so that in the ripe seed a central corrosion cavity is found. Packed in this is a coiled bundle of tubular structures (fig. 4). When these tubes are extended they measure about 23 mm. They have thick walls of a gelatinous nature. In *G. Gnemon* and *G. funiculare* no such coiled bundle of suspensors is found; they soon separate from each other and branch widely through the endosperm. Toward the tip of the suspensor in *G.* sp. 15, *G.* sp. 29, and *G.* sp. 59 the protoplasm becomes denser. It surrounds a peculiar, elongated, pear-shaped cell with a deeply staining nucleus and granular protoplasm. The apex of the cell is closely applied to the wall of the suspensor (fig. 9). A short distance from this end cell there appears an extremely large nucleus with a dense nucleolus showing one or more dark colored globules (fig. 9*a*). Sometimes two similar but smaller nuclei, closely associated, may be observed before the end cell is differ-

entiated, one of which may function in the formation of that cell. There are no nuclei distributed along the suspensors.

The condition of the tubes just described is that found in the ripe fruit on the trees, and in many of the seeds on the ground. At the next stage the suspensors swell to about five times their original size, and sometimes curve about as in fig. 5. The end cell widens, and moving out from the surrounding protoplasm crowds into a protrusion of the gelatinous wall of the tube (fig. 10). Division takes place first into two cells (fig. 11); then each of these divides again, forming the four cells shown in fig. 12. In this preparation one of the walls is not visible. The cells continue to divide in an irregular manner, forming an ovoid group (fig. 13). Division continues with elongation of the basal cells. The result is the formation of a long ribbon of tissue (fig. 14) which, like the primary suspensors, is folded in the cavity of the endosperm. This secondary suspensor, usually measuring about 13 mm., is formed of rather long thin-walled cells with large nuclei. This multicellular ribbon is very different from the secondary embryonal tubes of Abietineae, and, on the other hand, is not found at all in *G. Gnemon*, according to BOWER and COULTER. At the basal end of this secondary suspensor a chain of smaller cells with denser nuclei is differentiated, which may function in the proliferation of embryos. At the apex of the secondary suspensor the cells are actively meristematic (fig. 15). When the suspensor has lengthened sufficiently, it is the rapid multiplication of this group of cells which forms the massive embryonic body.

This embryonic body differentiates in a manner similar to that described for *G. Gnemon* by BOWER (1). Fig. 22 shows the first stage as it appears externally. At the apex a conical projection is visible, surrounded by a thick ridge, from which the cotyledons develop. Fig. 23 shows the "foot" or "feeder" projecting from the side of the hypocotyledonary stem. In fig. 24 both hypocotyl and "foot" have elongated, the latter having exceeded the former. Growth continues as shown in fig. 25, and the radicle reaches the micropyle. After forcing its way out of the endosperm, the radicle turns downward. Then the hypocotyledonary stem grows rapidly and makes its way out of the endosperm also. Following this, the

cavity is filled in around the "foot," which remains in the seed as an absorbing organ, so that the condition shown in fig. 26 results.

Sections of these embryos show that internally a root tip is differentiated in a position shown by the triangle *r* in fig. 19. A definite epidermis covers the cotyledons, hypocotyledonary stem, and conical apex of the stem. After the procambium bundles have differentiated, the foot or feeder is formed as a lateral protuberance of the hypocotyledonary stem, into which there is a lateral extension of the tissues. The procambium bundles make a deep loop toward the apex. In sections of an embryo at the stage shown in fig. 21, conspicuous rows of cells, forming the laticiferous ducts, are observed. In the region of the root tip these cells are distinguished by their dense contents (fig. 27). Toward the apex they are much longer (fig. 28), but have straight end walls in which thickened portions were not observed. These correspond to the younger ducts in *G. Gnemon* figured by BOWER. Young sclerenchyma cells, with the characteristic paired nuclei, are also present in these sections.

Polyembryony

It has been stated by BOWER that polyembryony is the rule in *Gnetum Gnemon*, as in other members of the group. From the examples of it observed in *G.* sp. 15 and *G.* sp. 59 it is plain that these species are not exceptions. Some of the variations of it are represented by the diagrams in text-fig. 1. In *A* three of the four primary suspensors have developed extensive multicellular secondary suspensors measuring about 13 mm. These three have grown so closely together that at this length one has not established itself as the successful embryo, although the middle one is slightly in the lead. In *B* are shown two competing embryos keeping pace at a length of about 16 mm. The third, having separated from the others, has become aborted at a length of 6 mm., while a secondary suspensor has not developed from the other tube. In *C* only one primary suspensor has developed a secondary one. This one has already become quite massive in structure, indicating that it is to become the mature embryo. A variation of this is shown in *D*. Here two of the embryos have developed secondary suspensors, but one is stunted, so that it is quite evident which is destined to

become the successful embryo. A case of the development of several embryos from one suspensor is represented by *E*. One of the secondary suspensors has developed into a bulky structure with unusually large cells. Toward the apex of this tissue, a number of groups of meristematic cells appear which resemble normal embryos in their development. The formation of these embryos is shown

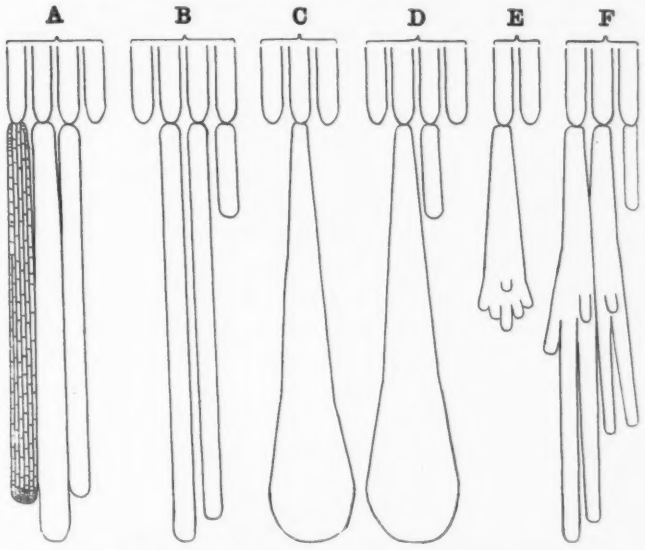

FIG. 1.—Diagrams illustrating polyembryony in *G.* sp. 15 and *G.* sp. 59: upper tubes represent primary suspensors; those below secondary suspensors; *H* and *D* represent usual type; *B* and *C* represent appearance found in small percentage of seeds; *E* and *F* show cases of proliferation of embryos, which are rarely observed.

in fig. 15, which is a small area of fig. 15*a* under greater magnification. The last diagram represents a branching of the secondary suspensor to form extra ones. In the preparation showing this condition the cells are indistinct, but the branches may be considered as prolonged "buds," such as those shown in *E*.

As already mentioned, chains of smaller cells may be observed in the tissues of the secondary suspensors. From these, in a number of cases, outgrowths have been observed which resemble a normal

embryo in development (figs. 6, 7). These cases of budding of embryos represent a process which, according to BUCHHOLZ (**2**), does not occur in the Abietineae. Also, although the secondary suspensors are often closely associated, no case was observed in which they fused to form a single embryo, a process described by BUCHHOLZ as normal in the higher Abietineae, but absent in the pine. From the material examined, it is concluded that in *G.* sp. 15 and *G.* sp. 59, as in other species of the genus as well as in *Ephedra*, (LAND **5**), although a number of embryos may begin to develop, only one reaches maturity.

Discussion

In respect to the long coiled bundle of primary suspensors, *G.* sp. 15, *G.* sp. 29, and *G.* sp. 59 are more like the Conifers than such species as *G. Gnemon*, in which the suspensors are widely separated in the endosperm. Furthermore, it is to be noted that, while in *G. Gnemon* the embryo develops directly on the end of the primary suspensor, in the other species studied a long multicellular secondary suspensor is produced, corresponding, although different in form and size, to structures present in the Abieteneae. In all species the development of the external form, as well as of the tissues of the embryo proper, is similar and resembles that described for *Welwitschia* (**4**).

In certain features of the gametophyte and endosperm THOMPSON has shown that *G. Gnemon* is quite different from the other species of the genus, and nearer the angiosperms. It is of course also distinct in its arboreal habit, for the others are all vines. The present study shows that it is different from several other species in the development of its embryo. No long secondary suspensor is formed, and the primary suspensors ramify widely through the endosperm. The reduction in the suspensors appears to bring it nearer the angiosperms. The great reduction in the amount of free nuclear division is a character which separates *Gnetum* widely from the lower gymnosperms. COULTER and BOWER differ as to whether there is any free nuclear division in the formation of the embryo proper at the end of the primary suspensor.

There is certainly none in this position in the other species studied by the writer.

BUCHHOLZ concludes that cleavage polyembryony is a primitive condition in the Abietineae. It is therefore interesting to note that certain species of *Gnetum* retain this condition. There is no fusion of suspensors resulting in a reduction of the number of embryos, such as BUCHHOLZ finds in the higher Abietineae and regards as an evolutionary development. The occasional formation of supernumerary embryos by splitting is evidently of no fundamental significance.

Summary

1. The proembryo of *G. funiculare* consists of a single cell from which suspensors emerge in different directions.

2. Cross-walls and nuclei are formed in these tubes, associated with the branches.

3. In *G.* sp. 15, *G.* sp. 29, and *G.* sp. 59 the suspensors form a coiled rope in a cavity in the endosperm; while in *G. funiculare*, as in *G. Gnemon*, they branch widely through the endosperm.

4. In the ripe seeds a peculiar cell is present at the end of the primary suspensor.

5. When germination begins in *G.* sp. 15, *G.* sp. 29, and *G.* sp. 59, a very long multicellular secondary suspensor is formed by division of this cell at the tip of the tube. In *G. Gnemon* no such body appears.

6. The development of cotyledons, root tip, and "foot" at the end of the secondary suspensor is described.

7. Polyembryony is the rule. Several of the primary suspensors usually form secondary suspensors. In some cases these are closely associated and develop equally for some time. In other cases one of the suspensors wins out at an early stage, and the others separate and become stunted. Occasionally the tip of the secondary suspensor divides into a number of branches. Branches from the side of a secondary suspensor are sometimes observed.

8. Except in the reduction of the amount of free nuclear division in all species and in the suspensors in *G. Gnemon* the development is gymnospermic.

To Dr. W. P. THOMPSON I am greatly indebted for his constant interest and advice, and also for all material used in this investigation.

UNIVERSITY OF SASKATCHEWAN
CANADA

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EXPLANATION OF PLATES XXXIX–XLI

PLATE XXXIX

FIG. 1.—*G. funiculare:* proembryo, showing suspensors emerging; *S*, suspensors; *pt*, pollen tube; semi-diagrammatic reconstruction; ×50.

FIG. 2.—*G. funiculare:* proembryo, showing variation of fig. 1; semi-diagrammatic reconstruction; ×50.

FIG. 3.—*G. funiculare:* branching of tubes; *oe*, outside of endosperm; connections in broken lines not determined with certainty; semi-diagrammatic reconstruction.

FIG. 4.—*G.* sp. 29: part of bundle of tubes measuring 23 mm.; ×25.

FIG. 5.—*G.* sp. 15: swollen tips of tubes turning about; ×25.

FIG. 5*a*.—*G.* sp. 15: group of nuclei from tube marked *X*; ×475.

FIG. 6.—*G.* sp. 59: embryo forming from chain of cells in secondary cells (*S*) of secondary suspensor; ×215.

FIG. 7.—*G.* sp. 59: branch of secondary suspensor; ×107.5.

PLATE XL

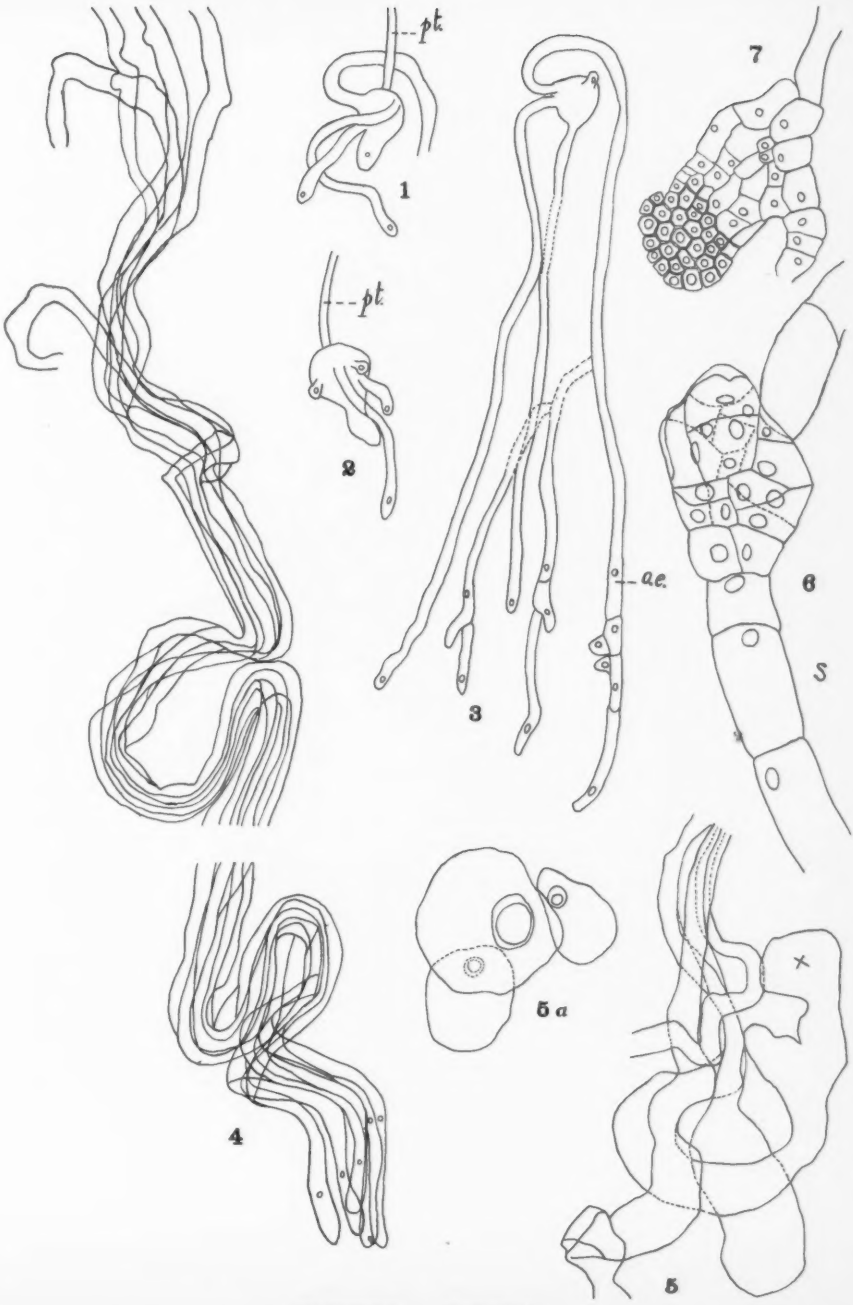
FIG. 8.—*G. funiculare:* branch of primary suspensor in endosperm; ×50.

FIG. 9.—*G.* sp. 29: tip of primary suspensor, showing peculiar cell; ×425.

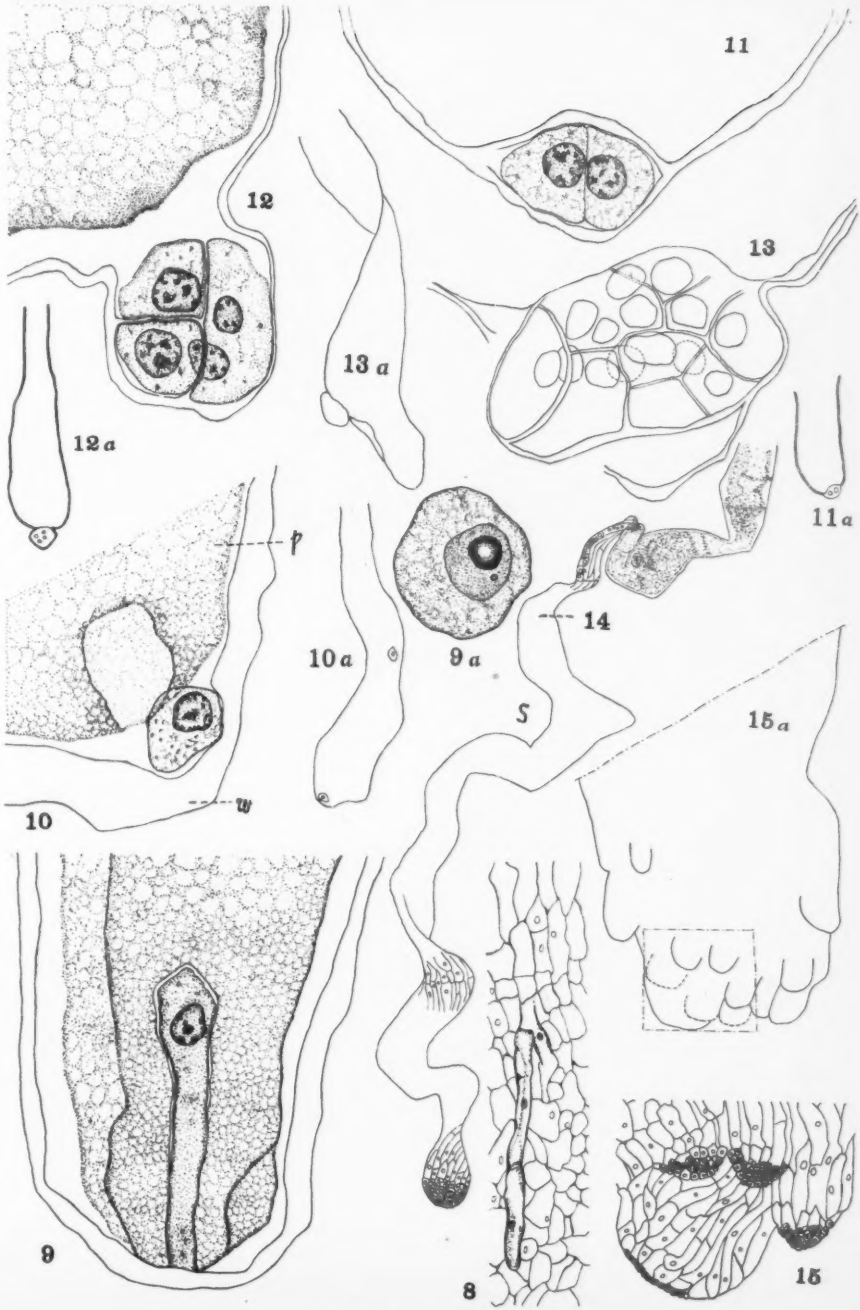
FIG. 9*a*.—*G.* sp. 29: large nucleus of suspensor shown in fig. 9; ×425.

FIG. 10.—*G.* sp. 59: shortened end cell moving out of surrounding protoplasm; *p*, shrunken protoplasm of tube; *w*, wall; ×425.

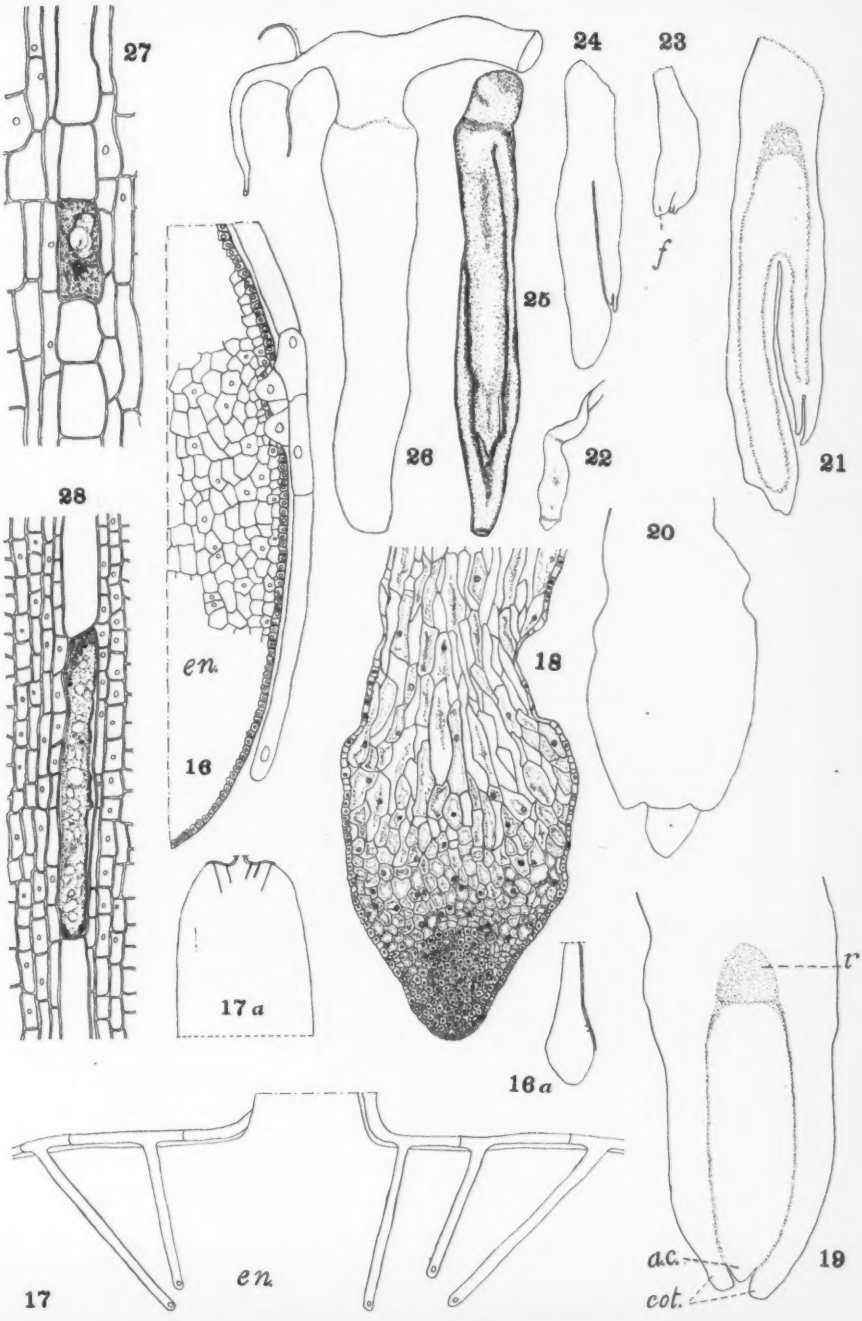
FIG. 10*a*.—*G.* sp. 59: same under lower magnification; ×50.



HAINING on GNETUM



HAINING on GNETUM



HAINING on GNETUM

FIG. 11.—*G.* sp. 15: 2-cell stage of secondary suspensor; tube placed to underlie these cells; ×425.

FIG. 11*a*.—*G.* sp. 15: same tube under lower magnification; ×50.

FIG. 12.—*G.* sp. 15: 4-cell stage; ×425.

FIG. 12*a*.—*G.* sp. 15: same tube under lower magnification; ×50.

FIG. 13.—*G.* sp. 59: group of cells irregularly formed; walls too faintly stained to see underlying ones; ×425.

FIG. 14.—*G.* sp. 15: swollen end of primary suspensor, showing ribbon of secondary suspensor; *s*, secondary suspensor; ×25.

FIG. 15.—*G.* sp. 59: groups of meristematic cells at the apex of secondary suspensor tissue; ×50.

FIG. 15*a*.—*G.* sp. 59: same under lower magnification, showing location of groups; ×25.

PLATE XLI

FIG. 16.—*G. funiculare:* suspensor outside endosperm, in immature seed, budding branches; semi-diagrammatic reconstruction; *en*, endosperm.

FIG. 16*a*.—*G. funiculare:* embryo sac, showing position of tube in fig. 16; semi-diagrammatic; ×5.

FIG. 17.—*G. funiculare:* branches penetrating endosperm from tangle of tubes without; in ripe fruit; diagram *en*, endosperm.

FIGS. 18–28.—*G.* sp. 15.

FIG. 18.—Section of tip of secondary suspensor, showing group of meristematic cells and structure of tissue; ×2.5.

FIG. 19.—Section of body of embryo showing stage before "foot" is differentiated; *cot*, cotyledon; *ac*, central apical cone; *r*, root tip; ×25.

FIG. 20.—Section of body of embryo; ×25.

FIG. 21.—Section of older embryo; ×7.5.

FIG. 22.—Embryonic body: gross material; ×7.

FIG. 23.—Embryonic body: cotyledons and "foot" (*f*) differentiated; gross material; ×7.

FIG. 24.—Same, later stage; ×7.

FIG. 25.—Same, later stage; ×7.

FIG. 26.—Root and hypocotyledonary stem outside of seed; ×7.

FIG. 27.—Longitudinal section of young laticiferous cells in basal part of embryo; ×107.5.

FIG. 28.—Longitudinal section of laticiferous cells from nearer apex; ×107.5.

A MORPHOLOGICAL STUDY OF CICER ARIETINUM

THEO. HOLM

(WITH PLATES XLII–XLIV)

Cicer arietinum has an interesting history; the generic name was proposed by PLINY, the specific by DODONAEUS, and both were accepted by LINNAEUS. Of the seven known species of the genus, *C. arietinum* is the only one of economic importance. It has been cultivated for many years, and at present it is not known in its wild state. According to DE CANDOLLE[1] it was evidently introduced from the Orient, and this writer has given an interesting account of its history. At the time of HOMER the plant was cultivated by the Greeks under the name "erebinthos"; by DIOSCORIDES it was called "krios," on account of the seed resembling somewhat the head of a ram; the Romans called it "cicer," from which the names "chiche" (Italy) and "pois chiche" (France) are derived; the name "kikere" is used by the Albanians. Although extensively cultivated in Egypt since the beginning of the Christian era, it does not seem to have been known to the ancient Egyptians. The meaning of the names used in Spain ("garbanzo" or "garbantzua") is uncertain, being neither Arabian nor Latin. In Sanscrit it is called "chennuka," and according to BRETSCHNEIDER the plant was cultivated in China in the fourteenth century under names indicating its introduction from the west. In Greece the dried seeds are salted and roasted, and are known as "stragalia" according to HELDREICH.[2] In our western states the seeds are used for coffee, hence the name "coffee-pea." For several years I have grown the plant in my garden at Brookland, D.C. Although it is a member of the Vicieae, it shows a very peculiar habit, and the structure is interesting in several

[1] DE CANDOLLE, ALPHONSE, Origine des plantes cultivées. Paris. 1886.

[2] HELDREICH, TH., Die Nutzpflanzen Griechenlands. Athens. 1862.

respects. No mention of this plant is made by SOLEREDER in his comprehensive work *Systematische Anatomie der Dicotyledonen*.

C. arietinum is an annual, branching from the base, the several stems erect, angular or winged, densely glandular-pubescent, as are also the leaves; the primary root is quite strong, and the lateral roots bear tubercles. The leaves are odd-pinnate with obovate, dentate leaflets (fig. 4), and the stipules are incised (fig. 6). The solitary flowers (figs. 4, 5) are white, with the wings free, and they are borne on axillary peduncles, strongly reflexed; the two prophylla are very distinct. The sessile ovary contains one or two ovules, and the filiform style is incurved. The pod is relatively large (fig. 6), ovoid to oblong, turgid, 2-valved; the seeds (fig. 7) are subglobose with the radicula almost straight. As stated, the plant is glandular-pubescent, and the hairs are clavate and pluricellular (fig. 8). They contain free oxalic acid, according to VAN TIEGHEM.[3]

Seedling

So far, no description has been given of the seedling stage of this plant. The seeds require only a few days (less than a week) to germinate; a young seedling is shown in fig. 1. The primary root (*R*) is quite long, vertical, and branches freely. There is no hypocotyl, and the cotyledons (*Cot*) remain subterranean, each with an axillary bud. The plumule develops an erect shoot, the first leaves of which are merely stipules. Fig. 2 illustrates an older seedling with the primary root still longer, and with the cotyledonary buds having developed into small shoots (fig. 3). The primary shoot now represents an erect stem with typical foliage, and several axillary branches.

It is characteristic of the seedling stage, therefore, that the cotyledons remain underground, subtending axillary buds; that the epicotyl (*Ep*) is erect and stretched; and that already the first stem leaves subtend branches. The glandular pubescence appears at the seedling stage, but is not shown in the figure, since the hairs cover the stem and leaves completely, and drawn in ink they would make the figures completely black.

[3] VAN TIEGHEM, PH., Traité de Botanique. Paris. 1884 (p. 542).

Internal structure of vegetative organs

ROOT SYSTEM

The roots are neither contractile nor developed as storage roots; they are simply nutritive and, although the plant is an annual, they increase quite considerably in thickness. This increase takes place by means of the activity of the pericambium, developing cork and a secondary cortex, beside by the development of cambial strata inside the stele, in the manner typical of dicotyledons. When fully matured the roots are very strong, owing to the abundance of stereïds outside the primary as well as outside the secondary leptome, and in the secondary hadrome. The primary structure may be studied from the apical portion of the primary root of the seedling. The epidermis is hairy, but there is no exodermis, and the cortex represents a compact parenchyma of about ten layers without deposits of starch. The endodermis is thin-walled, with Casparyan spots plainly visible. The pericambium consists of a single continuous stratum, but is separated from the stele proper by a layer of thin-walled parenchyma. Inside this parenchyma are four strands of stereome with leptome on the sides and on the inner face, beside four rays of hadrome, a small pith occupying the center of the stele. With regard to the hadrome, the protohadrome vessels are annular and reticulated, mostly two side by side, and much narrower than the inner, which vary from reticulated to porous. At this stage there are no signs of cell division in the pericambium, but narrow arches of cambial strata appear between the leptome and hadrome, and none outside the protohadrome vessels. The primary root is thus tetrarch, and the increase in thickness commences by the development of cambium between the leptome and hadrome.

Examining this same root in its older portion near the base, the following structure may be seen. Fig. 9 shows a diagram of the stele and part of the peripheral tissues, of which epidermis, cortex, and endodermis exhibit the same structure as described. Concerning the pericambium there is now a slight indication of increase, demonstrated by a tangential division (but only one) in each cell. The stereome is now more thick-walled (fig. 10, *St*), and there are many layers of cambium on the inner face of the leptome, also

outside the protohadrome vessels, giving rise to several strata of thin-walled parenchyma. This parenchyma formed outside the four strands of hadrome becomes the first medullary rays (fig. 11, *M*). This more advanced stage is readily seen in fig. 9, where the cambial strata (*Camb*) are very conspicuous, beside the commencement of the formation of the four medullary rays outside the vessels. The pith remains unchanged, without becoming sclerotic, and contains no deposits of starch. A corresponding structure occurs in the lateral roots, borne on the primary, but the cambium is not so abundant. If we examine the primary root of a mature fruiting specimen, we observe the structure as shown in fig. 11. All the peripheral tissues from epidermis to endodermis inclusive are lost, but replaced by several strata of thin-walled, homogeneous cork (*Co*), and a secondary cortex (*C*) in which the four stereomatic strands are yet distinct. The cork and the secondary cortex are the products of the cell division within the pericambium. Furthermore, the deep medullary rays may be seen, only two of which have been drawn, and these (*M*) proceed from the old hadrome rays (*PH*), where they were formed originally. Secondary medullary rays are also developed. They commence within the secondary hadrome, and are shorter and much narrower than the primary. The stele is now much broader, and contains secondary stereome (fig. 12, *St*) as cells scattered outside the leptome, also among the secondary vessels (fig. 14, *St*). On the other hand, the central portion of the stele, with the four rays of hadrome and the pith, are unchanged, while the primary leptome has become absorbed completely. These secondary formations within the stele, that is, the medullary rays, the stereome, the secondary leptome (*L*), and hadrome (*H*), are all products of the cambium (fig. 9).

Characteristics of the roots of *Cicer*, therefore, are the abundance of stereids; also, that the increase in thickness commences within the stele proper and not in the pericambium. The fact that a cambium becomes developed as a circular band between the secondary leptome and hadrome (fig. 11, *Camb*) shows that further increase in thickness is secured in exactly the same manner as in the collateral mestome strands of a dicotyledonous stem.

STEM

The stems are angular to narrowly winged, densely covered with glandular (fig. 8), long, unicellular, and pointed hairs; the glandular hairs contain free oxalic acid, according to VAN TIEGHEM. The stem structure in general is quite firm, owing to the presence of hypodermal collenchyma and pericyclic stereome. The cuticle is thick, longitudinally wrinkled, and the outer cell wall of epidermis moderately thickened (figs. 16, 17, *Ep*). Corresponding with the angles or wings are hypodermal strands of thick-walled collenchyma (fig. 17, *Coll*), and the cortical parenchyma is compact, about three layers, filled with chlorophyll. There is no endodermis, but a distinct continuous pericycle, which forms arches of stereome, but is only distinct in the older portions of the stem. In young internodes (fig. 15) the pericycle is so thin-walled that it is barely distinguishable. There are about nine primary mestome strands, which are collateral, and separated from each other by meristematic strata in young internodes.

In this meristem secondary formations arise by means of an interfascicular cambium (fig. 16, *Camb*), which begins from the sides of the mestome strands. As shown in fig. 16, a small strand of leptome (*L*) is the first product within this meristem, and later on the cambium continues to develop secondary hadrome, that is, porous tracheids and libriform (fig. 18, *H*). The pith is thin-walled, and not starch bearing, but large spheric crystals were observed, the material having been preserved in alcohol. Similar crystals also occur in the cortex of the old internodes. While the structure of a young internode shows no secondary formations, in the mature stem a compact stele is found in which thick-walled libriform is very conspicuous, developed from the interfascicular cambium.

Finally may be mentioned that the epicotyl of the young seedling is glabrous, with a smooth cuticle, but without collenchyma. The cortex is very thick, about twenty layers, and an endodermis is developed outside twelve arches of pericyclic stereome, corresponding with twelve collateral mestome strands. No secondary formations occur at this stage.

Characteristic of the stem structure of *Cicer*, therefore, is the presence of endodermis in the epicotyl, and its absence from the internodes above; the glandular hairs, very seldom met with in this family; the presence of interfascicular cambium; and the spheric crystals in the cortex and pith.

LEAF

The leaf structure is not exactly bifacial, since the stomata are distributed over both faces, and equally abundant; but the chlorenchyma shows both palisade and pneumatic tissue. Hairs like those of the stem abound on both faces of the blade, and the cuticle is wrinkled above and below the stronger veins; otherwise it is thin and smooth. The epidermis is slightly thick-walled above and below the midrib, and the lateral walls are undulate on the dorsal side (fig. 19), but almost straight on the ventral side. The stomata (fig. 19) have no subsidiary cells; they are free and raised a little, with a wide shallow air chamber. The chlorenchyma covers both faces of the leaf, and consists of a ventral palisade tissue of three layers (fig. 13, *P*), and of a pneumatic tissue of about five strata (fig. 13, P^+). There is neither collenchyma nor water storage tissue, and the veins are completely imbedded in the chlorenchyma. Around the midrib and the strong secondary veins are parenchyma sheaths, each cell of which contains a large rhombic crystal of calcium oxalate. Moreover, the midrib has a little pericyclic stereome on the leptome side, and consists of a single collateral mestome strand, with leptome, cambium, and a few vessels.

Characteristic of the leaf structure, therefore, is the distribution of the stomata over both faces of the blade; the dense chlorenchyma; the poor development of mechanical tissues; and the crystal bearing parenchyma-sheath. The internal structure of *C. arietinum* thus resembles that of a xerophilous plant among the Papilionaceae, especially when we add the profuse development of hairs, pointed, clavate, and glandular. The species evidently originated in a country with a warm and dry climate, and it has been suggested as possibly between Greece and the Caspian Sea.

CLINTON, MD.

EXPLANATION OF PLATES XLII–XLIV

PLATE XLII

FIG. 1.—Young seedling: *R*, primary root; *Cot*, cotyledons; *Ep*, epicotyl; natural size.

FIG. 2.—Older seedling; natural size.

FIG. 3.—Part of same, showing shoots (*S*) in axils of cotyledons; natural size.

FIG. 4.—Floral shoot; natural size.

FIG. 5.—Flower seen from below; natural size.

FIG. 6.—Pod; natural size.

FIG. 7.—Same in longitudinal section; natural size.

FIG. 8.—Glandular hair; ×480.

PLATE XLIII

FIG. 9.—Cross-section of young root: *Ep*, epidermis; *C*, cortex; *End*, endodermis; *St*, stereome; *L*, leptome; *Camb*, cambium; ×112.

FIG. 10.—Part of same root: *P*, pericambium; other letters as given; ×744.

FIG. 11.—Cross-section of old root: *Co*, cork; *M*, medullary ray; *PH*, protohadrome; *P*, pith; ×90.

FIG. 12.—Part of same root; ×744.

FIG. 13.—Cross-section of leaf: *Ep*, ventral; *Ep*$^+$, dorsal epidermis; *P*, palisade tissue; *P*$^+$, pneumatic tissue; ×480.

PLATE XLIV

FIG. 14.—Part of root: letters as in fig. 11; *V*, vessels; ×744.

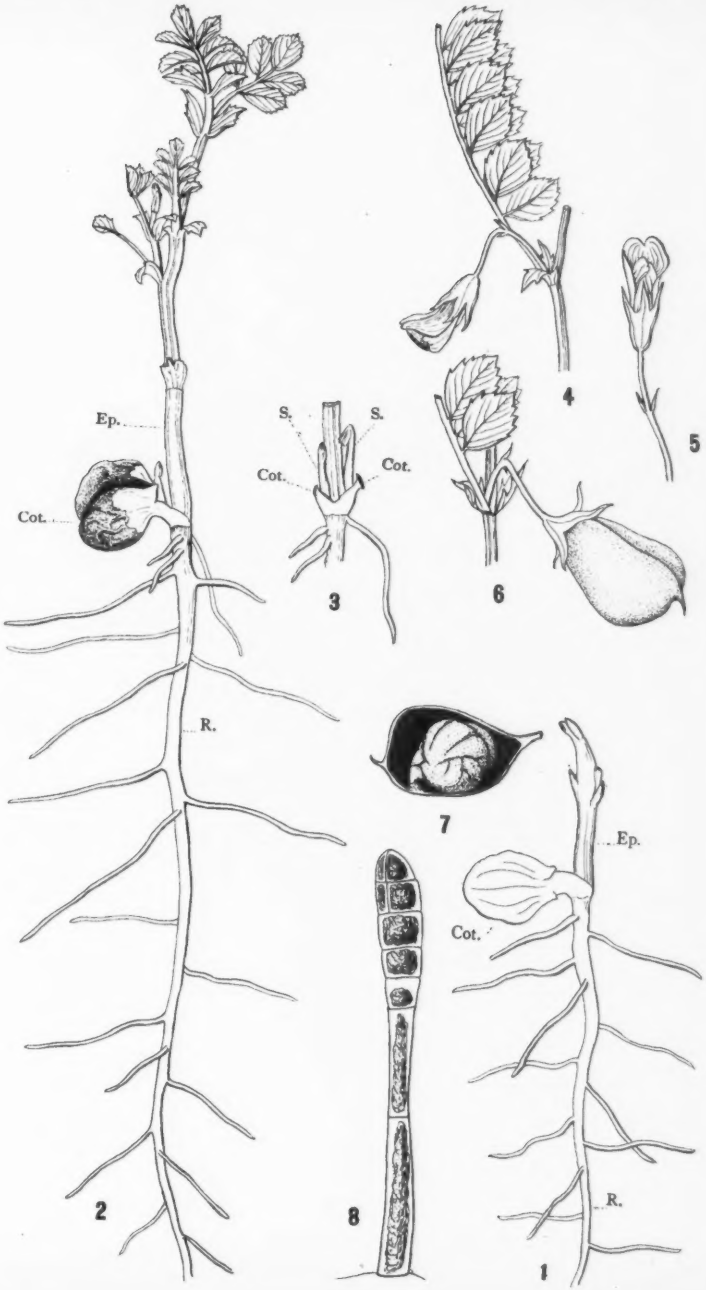
FIG. 15.—Cross-section of stem; ×480.

FIG. 16.—Part of same: *L*, leptome above interfascicular cambium (*Camb*); ×744.

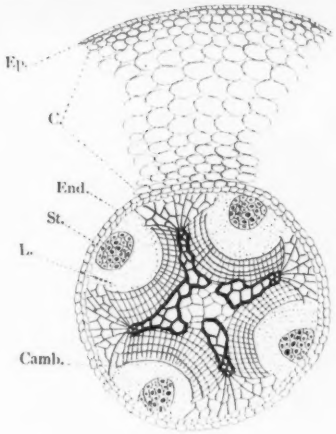
FIG. 17.—Part of same: *Coll*, collenchyma; ×480.

FIG. 18.—Part of same: all tissues of secondary formation; ×480.

FIG. 19.—Stoma of leaf; ×480.



HOLM on CICER



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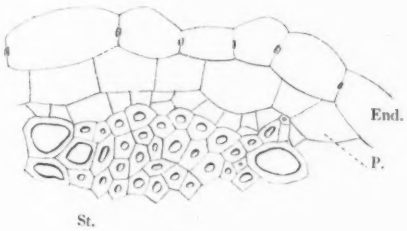

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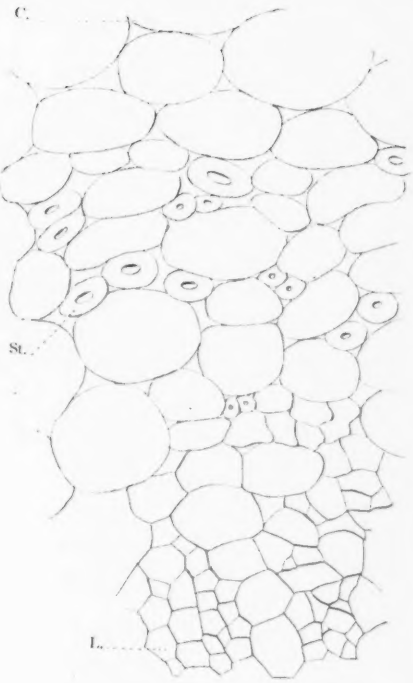

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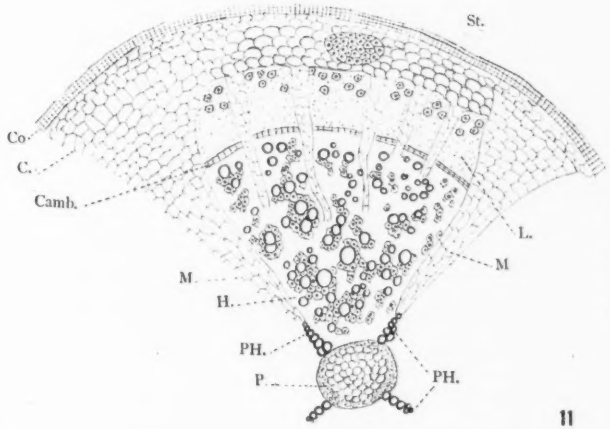

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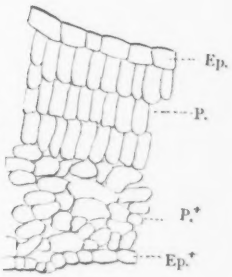

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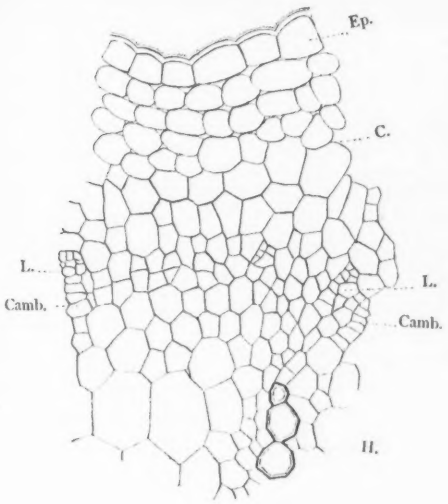

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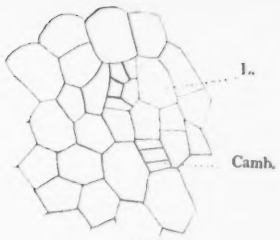

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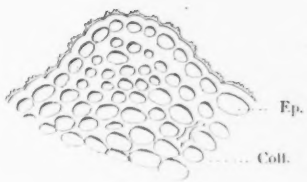

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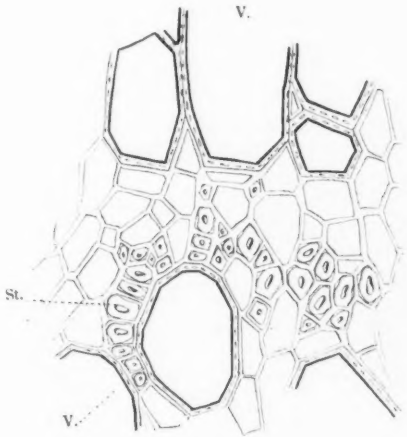

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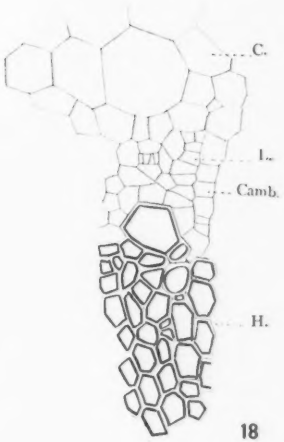

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HOLM on CICER

CORRELATION WORK IN PEAT-LAND PROBLEMS

A. P. DACHNOWSKI

A detailed account has recently been published by the writer showing for the first time the different types of plant organic material which are found in peat deposits within the glaciated area of this country.[1]

It was pointed out that this aspect of the peat problem had a practical importance for the close correlation which exists between the botanical composition of the different types of peat material and its corresponding physical, chemical, bacteriological, or other merits. Research of preliminary character along these lines on at least two distinct types of peat was reported in 1912.[2]

More extended field work, including peat in the southern states and the Pacific Coast, not only has confirmed that further research in these directions would be very desirable, but emphasized that it is precisely this information upon different types of peat material which supplies the essential criteria concerning the development and structure of peat deposits. Moreover, the facts are of fundamental importance also in the selection of workable tracts and in questions relating to the best means and practices of utilizing this source of national wealth for intensive or diversified agricultural and industrial purposes.

Peat deposits afford a large and profitable scope for a combination of scientifically directed industrialism and agriculture. They are an important national asset for fuel and power, for food and for certain products, and their best development will come when industries move to or near well-chosen, interconnected accumulations of peat, in order to insure a regular and continuous supply of different materials and to meet the demands of different markets. A great advantage would result from a better understanding of peat

[1] DACHNOWSKI, A. P., Quality and value of important types of peat material. U.S. Dept. Agric., Bull. 802, 1919.

[2] ———, Peat deposits of Ohio. Geol. Survey Ohio Bull. 16., pp. 424. *pls. 8, figs. 29.* In cooperation with the U.S. Bureau of Mines, 1912.

deposits and what they mean as raw materials to certain industries and to the investigators themselves.

Whatever the purpose for which a peat deposit may be used, it should clearly be understood that individual deposits of peat material present considerable variation, both in structure (the relative position of different layers) and in content (the character and quality of the different types of plant remains). All deposits are not the same; they will not yield to the same treatment, and they do not have the same value for scientific studies or for crops and for manufacturing purposes. It is largely on account of the failure to recognize these differences in stratification and in the quality of the peat materials that much of the scientific work in chemical analyses, in fuel and fertilizer determinations, in absorption and other data in the field of American peat investigations does not meet practical requirements. Inability to select suitable peat materials and workable deposits has made the peat-land problem difficult and uncertain. Crop yields and manufactured peat products have been unsatisfactory in many cases because definite information is not available as to the character of peat deposits in the United States, their actual acreage, and distribution. The data are still lacking on which to base the percentage of peat-land now in use and the real value of the unused areas of peat-land in this country.

This situation is significant in the efforts now being made to use these resources for fuel. It is no doubt possible, in most cases, to extend the agricultural uses of peat deposits and at the same time to meet the industrial needs with suitable deposits for centralized power stations or other manufacturing interests. At present these problems are not being met with the aid and cooperation necessary for various sections of the country. A comprehensive national program or policy of peat-land utilization may now be formed with safety. It should provide for the present and future needs of peat problems, for the conservation of unworkable deposits of peat, and for the best methods of combining agricultural and industrial interests where conditions favor the production of fuel and finished peat products as well as the demand for food. Furthermore, the basic principles and characteristics governing the utilization of peat deposits should be set forth for the

education of those concerned with the practical management of peat-lands. It is this phase of the problem concerning which the least has been done.

The basic importance of the stratigraphy of peat deposits and of development methods and principles in the interpretation of plant remains, such as peat deposits represent, has not as yet been generally recognized in this country. The investigator who views products and processes from the genetic and correlational standpoint has not become as prevalent in the field of peat investigations as one might surmise, and naturally there is still wanting the proper understanding of the profile structure of deposits which would make possible a satisfactory coordination of scientific activities as well as the effective agricultural or industrial use of specific peat-land areas. The scientific as well as the economic consequences of peat deposits under utilization are only made clear by a knowledge of the structure of the deposits. Not only commercial considerations but also the grasp of past and present modifying field conditions, the habit of regarding peat deposits from the broader scientific, even though at times rather theoretical, point of view will further and extend the possibilities of peat-lands.

To obtain information on yields and cost of production, or pertaining to experimental work which will permit interpretation of results, there must be more definite knowledge than is available now in regard to peat deposits and their materials. There is need, among other things, of a comparative study of the structural features of American and European peat deposits. The areas selected should comprise the latest and most authoritative investigations of workers in peat problems. The aim should be to state the stratigraphic facts fully as the data available permit, and it should include views, correlations, and, where the scope of the work warrants, advice on matters pertaining to investigations or securing information in the general field of peat-land problems.

The method of procedure in comparing profile features of peat deposits should be based on the botanical composition and physical appearance of the layers of peat. These furnish the information that strata of a certain type occur in certain localities on the American and European continents; that they replace one another, the

later ones being superimposed upon earlier layers; that they were formed in the course of a characteristic sequence or succession of vegetation units; and that the layers are more or less evidently connected with responses to changes in basic habitat factors.

It is of great importance to realize the influence which structural differences in the peat deposits exert upon the progress of peat-land agriculture, upon the advancements in the peat fertilizer industry, and upon improvements in mechanical devices for excavating, pulping, drying, or converting peat into fuel and other products.

Peat investigations in this country have reached the stage where basic correlations are possible between pleistocene geology, the distribution of peat deposits and their post-glacial vegetation units, and the climatic factors which in the past controlled the development and structure of peat accumulations. Clements[3] has recognized the need of extending peat investigations into the past, correlating geology, climatology, and the migration of former plant populations. A complete study of peat deposits is no longer possible without the aid of other sciences. It will now be practicable to extend European investigations dealing with climatic changes to the morainic systems of North America and to show whether or not glaciations have been contemporaneous, whether they depended upon general or local causes, and whether plant populations have immigrated and were affected by alternating dry and humid periods.

The information upon the different types of peat material offered in Bulletin 802 will aid in a tentative way, it is hoped, toward a solution of various peat-land problems. There are numerous questions in physical, chemical, and bacteriological studies, and also in physiological investigations dealing with peat materials which can now be attacked more successfully from this new standpoint.

The improvement of the present situation in matters of drainage, the management and the general uses of peat deposits for agriculture and for technical industries, should become obvious if consideration is given to the structural differences of deposits, especially to those deposits where systematic field experiments are to be carried out or various lines of practices are to be tested. Peat-

[3] CLEMENTS, F. E., Plant succession: An analysis of the development of vegetation, pp. 512. Carnegie Inst. Washington, publ. 242. 1916.

land differs very much from that of mineral soils. The bulk of a peat deposit is water and must contain not less than 65–70 per cent of water to be serviceable for the growth of crops. Moreover, in the great manufacturing states, particularly of the east and south, workable peat deposits are often involved which have a considerable acreage. On account of the fact that drainage and the utilization of such areas must frequently ignore state lines and control canals, dams, and the use of water for transportation or for irrigation purposes, the lines along which production should arise within certain selected areas of peat call not only for policies and organization, but also for extensive operations and for the collective working and association of small holdings into larger units.

It is not too late to adopt a national policy with regard to the conservation and utilization of peat deposits that are too large for individual enterprises, or which have been reserved for colonization. The only safe course is to determine carefully the character of the peat-land in the various regions of this country, and to lay a foundation for the methods and practices which will convert suitable land into productive areas. This policy of aiding and cooperating with the several interests should have national importance as well as state and local significance.

The relatively small quantity of plant remains in any peat deposit with a 65–70 per cent water content has not only unusual physical properties, but it contains various groups of organic compounds of great technical importance, and provides also the culture medium for bacterial organisms by means of which the organic matter may be made invaluable to agriculture. Peat materials are usually deficient in mineral salts suitable for the growth of cultivated crops, and they are not well balanced in fertilizer constituents. It is superfluous, therefore, to point out that the burning of organic material to increase the productiveness of the land is an erroneous practice which should be strongly condemned. As a general policy extensive drainage projects far in advance of the actual utilization of the peat deposits concerned should not be regarded as economical.

Engineers in various professions, after a careful study of peat-lands in relation to the nitrogen problem and the question of central electric power stations, have arrived at the conclusion that we are

only on the threshold of the great returns which workable peat deposits hold out to the industries combined with agricultural production. Until field work and experiments in this country begin to be conducted systematically on peat deposits, however, with a well-understood profile structure, and provision made for complete and continuous records, the methods and the results obtained in Germany, Sweden, and Holland will remain the chief sources of information and of practice. A critical and comparative study of the structural features and field conditions of American peat deposits is alone decisive. It is prerequisite in establishing the scientific foundation and the best practices which are necessary to successful utilization of peat-lands in this country.

BUREAU OF PLANT INDUSTRY
WASHINGTON, D.C.

ORIGIN OF MECHANISM OF HEREDITY

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 274

MERLE C. COULTER

The gross features of the mechanism of heredity have become features of general knowledge. The majority of biologists think of heredity in terms of determiners located upon the chromosomes. There are certain critical details of the mechanism, however, which still remain profoundly obscure. Little is known of the exact nature of the determiners themselves. The orderliness in the behavior of the determiners, that is, how they are "released" to express themselves only at the appropriate moments in the life history of the organism, seems not to have been clearly visualized. Finally, the possible origin of this mechanism of heredity is seldom even discussed. The present paper suggests, although only in a very brief and general way, certain answers to these questions.

It seems safe to assume that the most primitive organism lacked not only an organized nucleus, but even the components of a nucleus. A consideration of the activities of such an organism will reveal a suggestion as to the origin of the hereditary mechanism, provided, of course, that the assumptions are sound. The metabolism of this primitive organism, in certain fundamental features, will be similar to that of all organisms. Raw materials will be taken in and transformed to provide building materials and energy. If the raw materials be pure and the machinery of the protoplast perfect, this transformation will be complete, so that all the raw materials taken in will be transformed and used. Actually, however, the raw materials provided are never quite pure, and the machinery of the protoplast, although infinitely more efficient than any man-made machine, must be subject to certain flaws and frictions of its own. The transformation and use of materials, therefore, will not be complete; certain waste materials and by-products will remain. We are not concerned with the waste material; it is the fate of the by-products which is significant.

Certain of the by-products may be insignificant in their influence upon the protoplast. Others will undoubtedly be toxic in their effect, as many investigations upon auto-intoxication have gone to show. Primitive excretory systems, developed primarily for the disposal of waste materials, may remove a considerable part of these by-products. Thorough cleansing of the protoplast by this method, however, is impossible. Inevitably by-products will accumulate with the age of the organism. In fact age itself, in other than the purely chronological sense, is probably accounted for by this very accumulation of by-products. The toxic influence of these by-products will interfere with the efficient working of the machinery of the protoplast, and metabolism will be slowed down; hence "old age." Rejuvenescence occurs with cell division, because at cell division the protoplast is cleansed of many of these toxic by-products. This cleansing probably involves both physical and chemical forces. Physical reorganization at cell division will explain the exposure of these by-products; chemical oxidation will account for their removal (as toxins).

Again, were the machinery of this cleansing process a perfect one, rejuvenescence would be complete. Actually, however, the cleansing of the protoplast at cell division is not (or is not always) absolutely thorough. A few of the by-products pass over to the daughter protoplasts. The daughters, therefore, start life with a few by-products which the mother did not possess at the beginning of her life. Since these by-products are toxic and impair or retard metabolism, it is evident that the daughters are, at birth, slightly "older" than was the mother.

A series of repetitions of this performance through successive generations will have a cumulative effect. As a consequence, not only does the individual grow old through ontogeny, but, in a very real sense, the whole race is gradually aging through phylogeny. Evidence is not lacking that the higher organisms, cell for cell, have a lower rate of metabolism than do the more primitive ones. This is a statement of the quantitative effect of these by-products. It is their qualitative effect, however, that casts light upon the origin of the hereditary mechanism.

The by-products which originally accumulated in the protoplast were of various types. Some were very toxic, and these, if they were not immediately eliminated, resulted in the death of the organism. Others were less toxic, relatively more harmonious with the protoplast itself. These last, since they were not immediately fatal, stimulated an adaptive response on the part of the protoplast.

As to the general nature of this adaptive response, an important assumption must be made. Recent researches upon mammals have revealed in these organisms the power to develop antitoxins. The presence of a small quantity of toxin stimulates the organism to an adaptive response, the development of an antitoxin specific for the toxin present. This power is probably one of the fundamental characteristics of all protoplasm, being present even in the most primitive organisms.

Certain by-products in the primitive organism, only slightly toxic in their effect, stimulated it to produce an antibody. The protoplast is doubtless a colloidal system, and we may consider antibodies in the following light. The antibody counteracted the influence of the toxic by-product by insulating it from contact with the protoplast. Antibodies were probably developed most successfully for those by-products which were the least toxic in their effect. These by-products then became insulated by the antibodies. This insulation was significant not only in cutting off the influence of the by-product upon the protoplast, but in another respect also. At cell division this by-product, even though exposed, is not oxidized because of the protection afforded by the antibody which insulates it. It is probably this mechanism, for the most part, which accounts for the fact that some of the by-products are passed on to the daughter protoplasts, as mentioned before. These by-products are the primitive bearers of hereditary characters. The program carried out by the primitive hereditary mechanism is as follows.

The life of the primitive organism, like that of all organisms, involves a series of reactions. Early in the life of the organism there is present a certain reaction system, characterized by certain physical and chemical conditions. For a time the reaction system

as a whole maintains a sort of equilibrium, in which only reactions of a certain type are possible. This may be referred to as the *A* equilibrium. Inevitably, through the accumulation of certain materials, the *A* equilibrium will be upset. After a period of readjustment, the *B* equilibrium will succeed; this will be followed by the *C* equilibrium, and so on. The total number of distinct equilibria in the life program of any organism probably is in rough proportion to phylogenetic age.

Taking as an example the *X* equilibrium, certain questions may be considered. What is it that accounts for the existence of the *X* equilibrium? It is the inevitable result of reactions which took place during the *W* equilibrium. The *W* equilibrium may be similarly accounted for by the previous existence of the *V* equilibrium. The program is an inevitable one, and will be followed during each generation.

Under conditions imposed by the *X* equilibrium, only reactions of a certain type are possible, and these may be referred to as the *x* reactions. A number of *x* reactions are possible, x_1, x_2, x_3, etc. Chance conditions (environment, directly or indirectly) will determine which of these will take place. Whichever takes place, there will result a by-product, and this by-product will be of the *x* type. Even more specific than this, the x_3 reaction will result in and be characterized by the x_3 by-product. As the existence of the *X* equilibrium was inevitable, there will inevitably be laid down one of the *x* type of by-products. Since, however, it was chance which specifically selected the x_3 reaction, this same chance is indirectly responsible for the by-product x_3, rather than x_1, x_2, or any of the other possibilities.

The by-product x_3 will exist in an active state and exert some influence upon the protoplast so long as the *X* equilibrium continues. The eventual disappearance of the *X* equilibrium will be accompanied and characterized by the insulation of by-product x_3 by means of a specific antibody which has been developed by the protoplast. The *Y* equilibrium will follow; and, just as the *X* equilibrium was characterized by the free active existence of by-product x_3, one of the characteristics of the *Y* equilibrium will be the existence of x_3 in an insulated inactive condition. The by-

product x_3, insulated by its antibody, will pass on at cell division to the daughter protoplast. Early in the life of the daughter there must exist an A equilibrium. The inevitable program will then be followed, until finally the X equilibrium is reached. A very critical assumption is made at this point. The insulation of x_3 by its antibody is a phenomenon of colloidal chemistry. Similar colloidal reactions are known to be reversible. The formation of the antibody for x_3 took place at the inception of the Y equilibrium, which was characterized by the effective insulation of x_3. The X equilibrium, however, which now recurs during the following generation, is conducive to the free and active existence of any by-product of the x type. When the X equilibrium is reached in the life of the daughter protoplast, therefore, a dissolution of the antibody will occur and x_3 will be released.

With the X equilibrium now in existence, it is certain that reactions of the x type will take place. Which one of the possible x reactions occurred was in the first generation a matter of chance. In the present instance, however, the presence of by-product x_3 will exert a determining influence. The result, eliminating external stimuli of an unusual intensity, will be that the x_3 reaction and the x_3 by-product again stimulate the protoplast in a characteristic manner, developing in the daughter the same characteristic that was present at a similar stage in the life of the mother.

This theory accounts for the origin of the hereditary mechanism in terms of by-products and antibodies which insulate them. These various antibodies must form an important constituent of "modern" chromosomes, but there must also be present some more stable and homogeneous framework.

The release of the determiners (by-products) at the appropriate moment is referred to phenomena of colloidal chemistry. It is an open question whether this release is reflected by visible changes in the chromosomes. If so, a given locus on a chromosome should be seen in a loose or "open" condition only during a brief phase of the life history. No doubt this point would be hopeless to ascertain in any very accurate way.

As for the determiners themselves, these are visualized as by-products of metabolism, chemically active substances. The

reaction system (for example, X equilibrium), which arises as the result of an inevitable sequence of events, determines what general type of reaction shall take place at a given phase in the life history. The by-product (for example, x_3) merely decides which of a number of possible reactions within this general type shall be the one chosen.

The origin of a given by-product was accounted for by chance environmental conditions. The environment referred to may well have been the external environment in the case of the simpler organisms, but must be the internal environment in the more complex. This seems also to provide sufficient basis to explain the small degree of inheritance of acquired characters that has been said to take place.

UNIVERSITY OF CHICAGO

BRIEFER ARTICLES

EFFECT OF ZINC AND IRON COMPARED WITH THAT OF URANIUM AND COBALT ON GROWTH OF ASPERGILLUS

It has been shown in a preceding publication[1] that treatment of Pfeffer's nutrient solution with calcium carbonate at an elevated temperature removes from the solution the last traces of iron, zinc, and probably the other heavy metals to a high degree. It has also been shown that Pfeffer's solution so treated supports but a minimal growth of *Aspergillus niger*, but that the addition of both iron and zinc results in a phenomenal increase in growth as measured by the dry weight formed. The evidence presented, while showing that the presence of zinc in the cultural solution is just as necessary for *A. niger* as the presence of iron, did not include the action of other heavy metal salts.

Theoretically the extension of these observations to include other heavy metal salts is of importance, inasmuch as some light would be thrown on the parts played by iron and zinc salts in the metabolism of this fungus. If other heavy metal salts can replace either or both iron and zinc salts, as is commonly stated, then iron and zinc are not essential for the growth of *A. niger* and play the part of "chemical stimulants." If they cannot be replaced by other heavy metal salts, then they possess properties that remove them from this category and place them among the elements essential for the growth of *A. niger*.

For the purpose of this comparison only $Co(NO_3)_2+6H_2O$ and $UO_2(NO_3)UO_2(NO_3)_2+6H_2O$, both Kahlbaum reagents, were available, and these were therefore used. The method used has already been described (see footnote 1).

The results with $Fe_2(PO_4)_2+4H_2O$ and with $ZnSO_4+7H_2O$ apparently point to an incomplete action of the treatment with calcium carbonate, especially in reference to the removal of iron, since the cultures to which only zinc was added gave dry weights much higher than usual (see footnote 1). In the series containing both iron and zinc the usual phenomenal increases in dry weight occurred. Also regarding acidity it will be noticed, what has already been pointed out, that a parallelism seems to

[1] STEINBERG, R. A., A study of some factors in the chemical stimulation of the growth of *Aspergillus niger*. Amer. Jour. Bot. 6:330. 1919.

TABLE I

EFFECT ON GROWTH OF *A. niger* OF ADDITION OF IRON, ZINC, COBALT, AND URANIUM SALT TO TREATED PFEFFER SOLUTION (STRAIN W, 12)*

SOLUTION	YIELD	PH	SPORULATION
		$FePO_4$	
$FePO_4$/flask	(gm.)		
0 mg	0.049	3–4	Fair
1	0.044	3–4	Fair
5	0.046	3–4	Fair
20	0.047	3–4	Fair
50	0.072	3–4	Fair
		$ZnSO_4+7H_2O$	
Zn/liter			
0 mg	0.026	3–4	Fair
1	0.060	3–4	Fair
5	0.061	3–4	Fair
20	(0.267)	2–3	Good
50	(0.362)	2–3	Practically sterile
		1.0 mg. $FePO_4$/flask + zinc†	
Zn/liter			
0.1 mg	0.377	2–3	Good
0.5	0.771	1–2	Fair
1	0.694	1–2	Fair
10	0.688	1–2	Fair
20	0.723	1–2	Fair
50	0.759	1–2	Fair
		1.0 mg. $FePO_4$/flask + $Co(NO_3)_2$	
$Co(NO_3)_2$/flask			
0.1 mg	0.072	3–4	Practically sterile
0.5	0.058	3–4	Practically sterile
1	0.053	3–4	Practically sterile
10	0.049	3–4	Practically sterile
20	0.033	3–4	Practically sterile
50	0.026	3–4	Practically sterile
		1.0 mg. $FePO_4$/flask + $UO_2(NO_3)_2$	
$UO_2(NO_3)_2$/flask			
0.1 mg	0.048	3–4	Practically sterile
1	0.062	3–4	Practically sterile
5	0.062	3–4	Practically sterile
10	0.058	3–4	Practically sterile
20	0.069	3–4	Practically sterile
50	0.080	3–4	Practically sterile

* The compounds used in the studies were Baker's "analyzed" KH_2PO_4; $ZnSO_4+7H_2O$; and $CaCO_3$; and Merck's "reageant" NH_4NO_3; $MgSO_4+7H_2O$ and "highest purity" sucrose. All other compounds were the Kahlbaum.

† The zinc ($ZnSO_4+7H_2O$) was added to these cultures 3.5 days after inoculation.

TABLE I—*Continued*

Solution	Yield	Ph	Sporulation
	0.1 mg. Zn/liter+Co $(NO_3)_2$		
$Co(NO_3)_2$/flask	(gm.)		
0.1 mg	0.075	3–4	Practically sterile
0.5	0.103	3–4	Fair
1	0.093	3–4	Fair
10	0.190	3–4	Excellent
20	0.180	3–4	Excellent
50	0.192	3–4	Excellent
	0.1 mg. Zn/liter+$UO_2(NO_3)_2$		
$UO_2(NO_3)_2$/flask			
0.1 mg	0.062	3–4	Practically sterile
1	0.053	3–4	Practically sterile
5	0.053	3–4	Practically sterile
10	0.138	3–4	Fair
20	(0.462)	2–3	Fair
50	0.095	3–4	Practically sterile

exist between increase in dry weight, increased acidity, and decrease in sporulation.

Examination of the data obtained with $Co(NO_3)_2+6H_2O$ and with $UO_2(NO_3)_2+6H_2O$ shows the surprising result that the phenomenal increases in dry weight ascribed to these metals do not occur.[2] The increases in dry weight, excepting for a few cases (zinc plus cobalt salts), are within the experimental error of the observations. The unincreased acidity of the cultures supports the viewpoint that no increases in growth had been obtained. As concerns sporulation, there seems to be no regularity in the cultures containing cobalt or uranium, and while it is interesting to note that in the last series, containing only cobalt and uranium, sporulation took place, it would be an error to suppose that this necessarily implies that neither zinc nor iron is needed by *A. niger* for spore formation. The probable explanation is the presence of traces of iron and of zinc in the cobalt and uranium salts.

The lack of any marked increases in dry weight brought about by the addition of cobalt and uranium salts to the cultural solution is entirely contrary to the results already recorded in the literature. The explanation apparently best fitting the facts is that partial replacement

[2] Le Pierre, C., Remplacement du zinc par l'uranium dans la culture de l'*Aspergillus niger*. Compt. Rend. **156**:156. 1179. 1913; Richards, H. M., Die Beeinflussung des Wachstums einiger Pilze durch chemische Reize. Jahrb. Wiss. Bot. **30**:665. 1897.

may occur and does occur only when iron and zinc are not present in too low amounts. It would seem certain, considering the many described cases of increased growth caused by heavy metal salts, that the contamination of the "stimulants" with iron and zinc could not have occurred without some exceptions if contamination of the "stimulants" were the cause.

It is not impossible that the action of iron and of zinc and the action of the other so-called "chemical stimulants" will be found to be intimately connected with the increased acidity of the nutrient solution. This hypothesis has already been discussed in a former paper. The assumption of a direct action of the "chemical stimulants" must be dropped, however, since these experiments make apparent that their effects are in some manner related to those of iron and zinc, which latter are directly correlated with acid production by the organism. This explanation can also be extended to include the alkaloids, since the extremely high concentrations in which they are effective in bringing about increased growth would eliminate from consideration their action as alkaloids, and their chemical properties as free base and as acid salts are well known. The action of the chemically inert "stimulants," such as ether, chloroform, etc., may in the same manner be laid to the disturbance of the respiration processes leading to an accumulation of organic acids. The fact that free oxalic acid, in common with other acids, can bring about this increased growth, etc., renders this supposition not improbable, in spite of the fact that it is commonly stated that the products of catabolism of an organism exercise a harmful effect on its growth.[3] Clearly we have here a case in which a product of the metabolism causes an acceleration of the rate of growth of the organism.

These studies must be looked upon, therefore, as a confirmation of the conclusions arrived at by RAULIN (Études chimiques sur la végétation. Ann. Sci. Nat. Bot. V. **11**: 93. 1869) and by JAVILLIER (La Présence et la rôle du zinc chez la plantes. Theses. Paris. 1908) that both iron and zinc are essential for the growth of *Aspergillus niger*, and the assumption must be made that when iron and zinc are present in favorable amounts a partial replacement of either or both these elements can occur by the so-called "chemical stimulants."—R. A. STEINBERG, *Bureau of Plant Industry, Washington, D.C.*

[3] PFEFFER, W., Physiology of Plants. I. Oxford. 1897.

CURRENT LITERATURE

NOTES FOR STUDENTS

Phragmospheres and binucleate cells.—BEER and ARBER[1] maintain that there is a binucleate phase during the development of the parenchymatous tissues of the higher plants, which is preceded, and usually succeeded, by a uninucleate phase. They conclude that the binucleate condition is invariably brought about by mitosis, and state:

> The division occurs normally in the earlier stages, up to the period at which the two daughter nuclei are at the poles of the spindle, while the cell-plate is just being initiated. But at this point the mechanism seems to break down and the cell-plate is resorbed, while the phragmoplast, with its associated cytoplasm, goes through a singular metamorphosis. It becomes vacuolate in the center and develops into a hollow sphere which gradually grows until it incloses both the daughter nuclei, and then, by its further extension, ultimately merges into the cytoplasm lining the cell wall. For this hollow shell we have proposed the term "phragmosphere." In some cases it is exceedingly well defined and stains deeply, giving the sections in which it occurs a curious appearance of exhibiting cells within cells.

The writer[2] recently has described certain cytological phenomena which appear to be significant in this connection. In longitudinally dividing cells of the cambium of the higher plants the central spindle expands laterally by the addition of peripheral fibers, and gradually assumes the form of a disk. The connecting fibers and later the accessory fibers thicken to produce a cell plate and then disappear, leaving a circular rim of kinoplasm. In tangential longitudinal sections of the cambium, this ring-shaped aggregation of kinoplasmic fibrillae, phragmoplast, forms a halo about the daughter nuclei and gives the impression of a "cell within a cell." It increases in circumference, by the addition of new peripheral fibers, until it intersects the radial facets of

[1] BEER, R., and ARBER, AGNES, On the occurrence of binucleate and multinucleate cells in growing tissues. Ann. Botany **29**:597–598. 1915.

———, On the occurrence of multinucleate cells in vegetative tissues. Proc. Roy. Soc. B. **91**:1–17. 1919.

ARBER, AGNES, Studies on the binucleate phase in the plant cell. Jour. Roy. Micr. Soc. March 1920. 1–21.

[2] BAILEY, I. W., Phenomena of cell division in the cambium of arborescen gymnosperms and their cytological significance. Proc. Nat. Acad. Sci. **5**:283–285 1919.

———, The formation of the cell plate in the cambium of the higher plants. Proc. Nat. Acad. Sci. **6**:197–200. 1920.

———, The significance of the cambium in the study of certain physiologica l problems. Jour. Gen. Physiol. **2**:519–533. 1920.

the protoplast. At this stage in its development the kinoplasm disappears from two sides of the phragmoplast, leaving two entirely separate aggregations of kinoplasmic fibrillae. These rod-shaped masses of kinoplasm (kinoplasmasomes) move in opposite directions, thereby extending the cell plate, until it eventually reaches the two ends of the protoplast. This type of cytokinesis is not confined to the cambium, but occurs in other somatic tissues, in elongated or much flattened cells whose planes of division have one long and one short dimension.

A comparative study of cytokinesis in different somatic tissues and in cells of different shapes and sizes indicates that the various types of cell plate formation, described by TREUB, STRASBURGER, SCHÜRHOFF, and the writer, are but different phases or stages of a single fundamental type of cytokinesis. The particular expressions of the phenomenon which may occur in a given cell are dependent upon the dimensions of the latter, its plane of division, and the size and location of the nucleus. Thus, in very small isodiametric cells, having a large centrally located nucleus, the cell plate quickly intersects the walls of the cell, without any extensive lateral growth of the phragmoplast. In larger elements there is sufficient room for the phragmoplast to attain the "halo" stage before the cell plate intersects the sides of the protoplast.

The striking similarity between BEER and ARBER'S figures of sections of "phragmospheres" and polar views of normal ring-shaped phragmoplasts, such as occur in the cambium and other tissues of the higher plants, raises the question whether the phenomena encountered by these investigators are not actually stages in the division of parenchymatous cells. Although they state that the whole phragmoplast with its associated cytoplasm becomes transformed into a hollow sphere which incloses the daughter nuclei, and that the cell plate is resorbed without forming a membrane, they present no conclusive evidence in favor of such a hypothesis. For example, they give no critical figures or detailed descriptions to elucidate various stages in the formation of the phragmospheres or the resorption of the cell plate. They state that the phenomena occur in cells which are not forming division membranes, yet the binucleate phase is admitted to reach its most characteristic expression in young active tissue, just previous to the maximum period of growth undergone by the region of the stem, leaf, or root in which it occurs. Of course, the fact that individual cells are increasing in diameter does not indicate necessarily that they have ceased to divide in a given plane. Although the material that I have studied is extremely favorable, owing to the large size of the cells, nuclei, and division figures and their symmetrical arrangement, I have frequently found it difficult to determine, in a given plane of section, whether cells contained more than one nucleus each. This is due to the fact that nuclei which appear to lie within a single protoplast, that is in the same focal plane, subsequently are found to be separated by thin, recently formed membranes or cell plates. BEER and ARBER'S conclusions seem to have been drawn largely from the study of transverse sections of stems and roots.

In view of the important bearing of BEER and ARBER'S conclusions upon various cytological and physiological problems, it is to be hoped that future investigations may throw some light upon the following questions:

1. Are BEER and ARBER'S phragmospheres actually hollow spheres of kinoplasm or ring-shaped phragmoplasts such as occur in other tissues of the higher plants?

2. If they are ring-shaped phragmoplasts, as seems probable, do they form cell plates?

3. Are the nuclei of the "binucleate cells" separated by a thin membrane, or does the cell plate disappear without forming a membrane?

4. Do the nuclei of multinucleate cells arise by the same type of nuclear division as the nuclei of binucleate cells?—I. W. BAILEY.

Hepaticae.—Among recent publications on the Hepaticae by EVANS are the following. In continuation of studies of the New England Hepaticae,[3] three species of *Nardia* are fully discussed, one of them being described as new. In continuation of the North American Hepaticae,[4] other species of *Nardia* are considered, also additions to the flora of the United States, extensions of range, and clearing up some difficulties in nomenclature. A taxonomic study of *Dumortiera*[5] contains a full discussion of the two species as to structure, classification, stations, and literature.

A new *Riccia*[6] (*R. bistriata*) from Peru presents a noteworthy feature in "the peculiar bands of thickening which are found in the walls of the green cells," a feature which has not before been noted in the Marchantiales. Three species of *Asterella*[7] from South America are presented as new combinations, transferred from *Fimbriaria*. They are not known to extend into North America; in fact, of the 15 North American species, only two are known to extend into South America.—J. M. C.

Ripening of tomatoes.—SANDO[8] finds that the maturity of a tomato is dependent upon its age and not upon its size in the growing conditions under which he worked. His analyses show that throughout the ripening period there is an increase in moisture, acids, and sugars, and a decrease in solids, total nitrogen, starch, pentosans, crude fiber, and ash. Sugars increase from

[3] EVANS, A. W., Notes on New England Hepaticae. XV. Rhodora **21**:149–169. *pl. 126. figs. 14.* 1919.

[4] ———, Notes on North American Hepaticae. VIII. Bryologist **22**:54–73. *pl. 2. figs. 15.* 1919.

[5] ———, A taxonomic study of *Dumortiera*. Bull. Torr. Bot. Club **46**:167–182. 1919.

[6] ———, A new *Riccia* from Peru. Torreya **19**:85–88. *fig.* 1. 1919.

[7] ———, Three South American species of *Asterella*. Bull. Torr. Bot. Club **46**:469–480. 1919.

[8] SANDO, CHAS. E., The process of ripening in the tomato, considered especially from the commercial standpoint. U.S. Dept. Agric., Bull. 859. 1920.

25.66 per cent in the fruit 14 days old to 48.32 per cent in ripe fruit. Starch decreases in the same period from 15.84 per cent to 2.65 per cent. Fruit picked after it had started to color and allowed to ripen in free access of air showed practically the same composition and edible qualities as fruit ripened on the vine. Commercially ripened tomatoes, due to lack of free access of air, show very different composition and inferior taste. Lack of ventilation during ripening increased the acid content 138 per cent and decreased the soluble carbohydrate content 21 per cent. Commercially ripened tomatoes wrapped with one paper showed similar but less marked changes. SANDO is unable to explain why wrapping with three papers was less detrimental than wrapping with one.—WM. CROCKER.

Seed sterilization.—BRAUN[9] has developed what he calls the "presoak" method of seed sterilization. "The use of formalin and copper sulphate as now practiced usually causes retardation and injury to seed germination." He finds that he can eliminate this injury by soaking the wheat seeds ten minutes and covering them for six hours to allow them to absorb the adhering water. Then follows the usual treatment with the disinfectant. The presoak method often shows a noticeable stimulative effect. BRAUN speaks of presoaking as saturating the walls and cells of the seeds so as to dilute the disinfectants beyond an injurious concentration. He also speaks of presoaking as increasing the efficiency of the disinfectants by bringing the dormant bacteria and fungi into a vegetative state so they are more easily killed by the disinfectants. —WM. CROCKER.

Plant distribution in South Africa.—An analysis of the plant population of South Africa by BEWS[10] shows that while WILLIS's "age and area" theory may be in general accepted, its application is not feasible in the region under discussion, on account of the very great climatic variations. Species widely distributed in South Africa are found to belong to pioneer stages of the various plant successions, although not all pioneer forms are widely distributed. Many species with a restricted distribution are shown to belong to climax associations, especially in the coast belt forest of Natal.—GEO. D. FULLER.

Studies in Taraxacum.—STORK[11] has extended our knowledge of this interesting genus, discovering "parthenogenesis" in additional species, in the sense that the unfertilized egg develops an embryo, but it is a diploid egg. He also records some interesting variations in the genus, and gives an account of megasporogenesis in *T. erythrospermum*.—J. M. C.

[9] BRAUN, HARRY, Presoak method of seed treatment: A means of preventing seed injury due to chemical disinfectants and of increasing germicidal efficiency. Jour. Agric. Res. **19**:363–392. 1920.

[10] BEWS, J. W., Plant succession and plant distribution in South Africa. Ann. Botany **34**:287–297. 1920.

[11] STORK, HARVEY E., Studies in the genus *Taraxacum*. Bull. Torr. Bot. Club **47**:199–210. 1920.

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